Energy balance, exercise-induced muscle damage, and the efficacy of nutritional interventions on recovery in female dancers.

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Energy balance, exercise-induced muscle damage, and the efficacy of nutritional interventions on recovery in female dancers.

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Abstract

It is well-documented that strenuous physical activity has the potential to elicit exercise-induced muscle damage (EIMD), particularly when the exercise is novel and has an eccentric component. Given that the symptoms of EIMD can compromise subsequent performance, there has been substantial investigation into potential strategies that might reduce these detrimental effects in athletic populations. However, while significant advances have been made in this field, few investigations address the diversity of exercising populations who might experience EIMD following the activities they engage in, and the strategies that could facilitate their recovery. Globally, dance and dance-based exercise are popular forms of recreational physical activity, and the intensity and volume of exercise previously reported in highly trained and professional dancers can often be comparable to that of many other elite athletes. The overall purpose of this thesis was to understand the nutritional challenges facing female dancers, increase knowledge of the EIMD response, and examine potential nutritional interventions to reduce the negative issues associated with damaging exercise in this understudied population.

The first study characterised the typical energy intake (estimated by combined 24 h recall and weighed food diary) and energy expenditure (estimated by the sum of basal metabolic rate, the thermic effect of food, and physical activity energy expenditure) of pre-professional contemporary dancers during 7 days of full-time training. This study determined that there is a prevalence of energy deficiency in this population (with an average daily deficit of -356 ± 668 kcal or -1.5 ± 2.8 MJ). Additionally, the second study demonstrated that female dancers experience EIMD, both from dance-specific and repeated-sprint exercise; with observed increases in muscle soreness, limb girth, plasma creatine kinase, and reductions in muscle function (all $p < 0.05$). These data served to inform the final two experimental chapters, which sought to investigate the role of nutritional interventions in alleviating the various symptoms of damage following repeated-sprint exercise in female dancers. Montmorency tart cherry supplementation accelerated recovery of countermovement jump height compared to placebo ($p = 0.016$). There was an improved recovery of reactive strength index ($p = 0.016$), flexibility ($p = 0.050$) and

reduced CK (p = 0.002) following supplementation with whey protein hydrolysate. Consequently, this research provides justification for the use of these supplements as practical interventions, which could be implemented to benefit the day-to-day life of a dancer; not least for promoting recovery, but also contributing to maintaining energy balance.

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The following abbreviations have been defined in the text in the first instance.

%BF	Percentage body fat
%CV	Percentage coefficient of variation
%TEI	Percentage of total energy intake
1RM	One repetition maximum
Akt	Protein kinase B
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BCAA	Branched-chain amino acids
BL	Baseline
BMI	Body mass index
BMR	Basal metabolic rate
Ca^{2+}	Calcium
CHO	Carbohydrate
CI	Confidence interval
CK	Creatine kinase
CMJ	Countermovement jump
COX	Cyclooxygenase
CRP	C-reactive protein
DJ	Drop jump
DLW	Doubly labelled water
DNA	Deoxyribonucleic acid
DOMS	Delayed onset muscle soreness
DP	Dance-specific protocol
EA	Energy availability
EB	Energy balance
EDTA	Ethylenediaminetetraacetic acid
EEE	Exercise energy expenditure
EIMD	Exercise-induced muscle damage
FFM	Fat free mass
FFQs	Food Frequency Questionnaires
FOXO	Forkhead box transcription factors
GM	Medial head of the gastrocnemius
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPX	Glutathione peroxidase
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
HR	Heart rate

hsCRP	High sensitivity C-reactive protein
IFN-c	Interferon-gamma
IFN-γ	Interferon-γ
IL	Interleukin
ISAK	International Society for the Advancement of Kinanthropometry
LDH	Lactate dehydrogenase
LIST	Loughborough Intermittent Shuttle Test
LOOH	Lipid hydroperoxides
LSD	Least significant differences
MC	Tart Montmorency cherry
MET	Metabolic equivalent
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
mTOR	Mechanistic target of rapamycin
MVC	Maximum voluntary isometric contraction
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
ORAC	Oxygen radical absorbance capacity
$_{P}$70S6K	Ribosomal protein S6 kinase beta-1
p-Akt	Phosphorylation of Akt
PAL	Physical activity level
p-FOXO1	Phosphorylation of FOXO class O1
PGE_2	Prostaglandin E2
PGH_2	Prostaglandin H_2
PL	Placebo
PLA_2	Phospholipase A_2
PPT	Pressure pain threshold
RBE	Repeated bout effect
RCF	Relative centrifugal force
RCTs	Randomised controlled trials
RF	Rectus femoris
RMR	Resting metabolic rate
RONS	Reactive oxygen/nitrogen species
RPE	Rate of perceived exertion
rpS6	Ribosomal protein S6
RSI	Reactive strength index
SC	Satellite cell
SD	Standard deviation
SEM	Standard error of the mean
SJ	Squat jump
SOD	Superoxide dismutase
SP	Sprint-specific protocol
SR	Sarcoplasmic reticulum

TAC	Total antioxidant capacity
TAS	Total antioxidative status
TBARS	Thiobarbituric acid reactive species
TEE	Total energy expenditure
TEF	Thermic effect of food
TEI	Total energy intake
TEM	Technical error of measurement
TFEQ-R18	18 item, 3-factor eating questionnaire
TNF-α	Tumour necrosis factor alpha
T-tubules	Transverse tubules
VAS	Visual analogue scale
VL	Vastus lateralis
$\dot{V}O_2$	Volume of oxygen consumption
$\dot{V}O_{2max}$	Maximal volume of oxygen consumption
WPC	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate

Publications

Peer reviewed publications arising from this course of investigation

Brown, M. A., Howatson, G., Quin, E., Redding, E., & Stevenson, E. J. (2017). Energy intake and energy expenditure of pre-professional female contemporary dancers. *PLoS One, 12*(2).

Brown, M. A., Howatson, G., Keane K., & Stevenson, E. J. (2016). Exercise induced muscle damage following dance and sprint exercise in females. *J Sports MedPhys Fitness, 56*(11), 1376-1383.

Adjunct peer reviewed publications during the course of investigation

Clifford, T., Allerton, D. M., **Brown, M. A.,** Harper, L., Horsburgh, S., Kean, K. M., Stevenson, E. J., & Howatson, G. (2016). Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Appl Physiol Nutr Metab,* EPUB ahead of print.

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Author's declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved and granted by the Faculty Ethics Committee.

I declare that the word count of this thesis is 44,713 words

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1 Introduction

The benefits of regular physical activity are irrefutable, with substantial evidence indicating enhanced cardiovascular, musculoskeletal, metabolic, and mental health (Warburton, Nicol, & Bredin, 2006). Despite this, exercisers, athletes, and sport and exercise scientists are faced with the potential for exercise to induce muscle damage, and the development of associated symptoms. There has been an increase in interest in exercise-induced muscle damage (EIMD) in the past twenty to thirty years. Initially, many investigations sought to determine the cause and mechanisms responsible for EIMD, and to define the symptoms and the time-course of recovery. It is now widely accepted that many forms of exercise, primarily eccentric-biased and novel exercise, can result in EIMD. This is characterised by the manifestation of symptoms including increased muscle soreness and pain, limb swelling, reduced range of motion, detriments in muscle function, increases in systemic indices of muscle damage, oxidative stress and inflammation, and compromised performance (Clarkson & Hubal, 2002). Arguably, of greatest concern is the duration of recovery and the implications that persistent symptoms have on subsequent exercise potential. Certainly EIMD may reduce motivation and compliance, not least of recreational exercisers to take part in recommended levels of physical activity for public health, but also of highly trained individuals and athletes who may be required to comply with particularly demanding training schedules. The ability to recover quickly from exercise is of great importance for many athletes and exercising populations that train or compete on single and consecutive days. By recovering quickly, in terms of restoring physiological and psychological indices to normal levels, it is anticipated that subsequent exercise performance is enhanced.

Owing to the considerable body of evidence demonstrating the detrimental effects of EIMD, interventions which may reduce these effects and accelerate recovery are highly sought-after. Consequently, the efficacy of many strategies has been investigated; for instance, the use of massage (Mancinelli et al., 2006; Shin & Sung, 2015), stretching (Chen, Chen, Jan, & Lin, 2015; Johansson, Lindstrom, Sundelin, & Lindstrom, 1999), cold water immersion (Eston & Peters, 1999; Goodall & Howatson, 2008), compression garments (Areces et al., 2015; Bieuzen et al., 2014), and non-steroidal anti-inflammatory drugs (NSAIDs) (Baldwin, Stevenson, & Dudley, 2001; McAnulty et al., 2007). However, perhaps the most common strategy investigated is the influence of nutritional interventions on EIMD and recovery

(Howatson & van Someren, 2008). Nutritional strategies which have demonstrated some efficacy include (among others) protein supplementation and their analogues, and more recently the use of functional foods that are rich in bioactive compounds. These may offer practical and natural alternatives to pharmacological drugs and analgesics which carry risks of suffering adverse effects (Ziltener, Leal, & Fournier, 2010).

For the most part, this research offers exercisers, athletes, and sport and exercise scientists the opportunity (and perhaps arguably the challenge) to identify interventions that could be applied to their own work. However, while significant advances have been made in this field, few investigations address the diversity of exercise modes which may be susceptible to EIMD, and the diversity of populations who would benefit from interventions to improve recovery. Indeed, it is recognised that there is large variability in the EIMD response among individuals and between exercise paradigms.

The characteristics of the exercise stimulus itself (the mode, duration, and intensity) can determine the degree of muscle damage. The majority of studies adopt extreme exercise paradigms for the purpose of eliciting a large EIMD response, presumably in order to increase the likelihood of observing treatment effects. Far fewer studies have examined EIMD following exercise-specific protocols, which can be applied to 'real-life' sport and exercise scenarios. For instance, dance and dance-based exercise are popular forms of physical activity, and the intensity and volume of exercise previously reported in highly trained/professional dancers (Twitchett, Angioi, Koutedakis, & Wyon, 2010) can often be comparable to that of many other elite athletes. In addition, dancers are often reported to consume low energy intakes, and the potential of this to influence EIMD cannot be ruled out. Yet little is known about the EIMD response and subsequent recovery elicited following dance and in dance populations. Moreover, there is a great deal of individual variability in the EIMD response; not least dependent on training status (Tee, Bosch, & Lambert, 2007; Ye, Beck, & Wages, 2015), but also dependent on sex. In particular, a number of properties of the female sex hormone oestrogen are thought to play a role in the reduced EIMD response often observed in females compared to males (Tiidus, 2000).

Collectively, the variability of EIMD makes interpretation and application of many research studies challenging, and may explain some of the contradictory findings that are evident in the literature. Indeed, a great number of questions regarding EIMD and recovery remain unanswered.

1.1 Thesis purpose and aims

Research in EIMD and recovery is largely conducted in male or mixed-sex populations and primarily employ exercise paradigms that lack ecological validity to specific athletic populations. In addition, there are many challenges faced by dancers, which warrant further research; not least the prevalence of energy deficiency, but also the expectation of maintaining high training loads with short periods of recovery. Nutritional interventions may therefore contribute to both restoring energy balance and assisting in exercise recovery in these populations.

In light of the limitations in the literature, the overall purpose of this thesis was to understand the nutritional challenges facing female dancers, increase knowledge of the EIMD response, and examine potential nutritional interventions to reduce the negative issues associated with damaging exercise in this understudied population. Specifically, this thesis had four main aims, which are systematically addressed in four experimental chapters;

1) To determine the typical training and eating behaviours of pre-professional female dancers;
2) To examine the exercise-induced muscle damage response to both dance-specific and repeated-sprint exercise in female dancers;
3) To investigate the influence of Montmorency tart cherry juice supplementation on exercise-induced muscle damage in female dancers;
4) To investigate the influence of whey protein hydrolysate supplementation on exercise-induced muscle damage in female dancers.

The null hypotheses associated with these main aims are as follows:

1) There will be no significant difference between energy intake and energy expenditure of pre-professional female dancers;

2) There will be significant differences in the exercise-induced muscle damage response following both dance-specific and repeated-sprint exercise in female dancers;
3) Montmorency tart cherry juice supplementation will have no significant influence on exercise-induced muscle damage in female dancers;
4) Whey protein hydrolysate supplementation will have no significant influence on exercise-induced muscle damage in female dancers.

2 Literature review

The following literature review will firstly summarise the evidence concerning energy balance in dance populations, and outline the measurement techniques employed to estimate energy intake and energy expenditure (section 2.1). This is then followed by a discussion of the scientific principles surrounding exercise-induced muscle damage, with a particular focus placed on the methods of assessment (section 2.2). In addition, a number of determinants of muscle damage which should be taken into account when examining the evidence will be discussed. Finally, a critical overview of literature pertaining to the use of tart Montmorency cherry juice and whey protein hydrolysate supplementation for recovery following strenuous exercise will be presented (section 2.3). Section 2.1 will provide specific focus to dancers, however given a lack of pertinent information regarding muscle damage and the aforementioned nutritional interventions in this population, literature from other athletic populations, specifically females where possible, will be examined in sections 2.2 and 2.3. Whilst this was not a systematic review or a meta-analysis, the Medline database was searched primarily to identify publications of interest (published until October 2016). Assessment of their appropriateness was made from the abstract, and the reference lists of these were also consulted for additional relevant publications. For section 2.1, key terms used were ‘energy intake’, ‘energy expenditure’, and ‘energy balance’, in combination with ‘dance’ and ‘dancer’. Only those articles which included measurement of both energy intake and energy expenditure were then reviewed in detail in sections 2.1.3 and 2.1.4. For section 2.2, key terms used included ‘exercise-induced muscle damage’, ‘muscle damage’, and ‘exercise recovery’. For section 2.3, key terms employed included ‘protein hydrolysate’, ‘whey protein hydrolysate’, ‘Montmorency tart cherry’, and ‘sour cherry’ in combination with ‘muscle damage’ or ‘exercise recovery’.

2.1 Energy balance in dance

2.1.1 Introduction

In dance populations, there is often an expectation to maintain an ultra-lean body type given the artistic requirements of dance. As with many comparable aesthetic sports, though extremely low body mass and fat mass are known to negatively

influence performance and recovery potential, low levels are nevertheless often considered to be advantageous for movement efficacy and artistic expression (Sundgot-Borgen & Garthe, 2011). Specifically, leanness is thought to facilitate dance partnering and allow dancers to be more energy efficient, physically articulate and agile, and aesthetically pleasing (Bonbright, 1989). As a result, maintaining a lean physique is thought to be an important aspect of dance fitness and a pre-requisite for success in the profession (Claessens, Beunen, Nuyts, Lefevre, & Wellens, 1987; Hergenroeder, Brown, & Klish, 1993). Indeed, it has been reported that dance teachers and artistic directors often demand low body weights (Calabrese et al., 1983) and dancers may be encouraged to lose weight (Cohen, Potosnak, Frank, & Baker, 1985). Elite dancers might be denied employment or study based on their physique (Sandri, 1993), and increases in body mass can even result in expulsion from a dance school (Kostrzewa-Tarnowska & Jeszka, 2003). This is likely to be exacerbated by the increase in the quality and quantity of professional dance schools and a concomitant increase in levels of competition (Bonbright, 1989). Consequently, it is perhaps unsurprising that weight and diet have been reported to be a primary concern of dancers (Sandri, 1993). In fact, being too heavy is suggested to be of more concern to dancers than the consequences associated with weight control behaviours (Sandri, 1993); which include fasting, excessive exercise, self-induced vomiting, and laxative abuse (Maloney, 1983; Robbeson, Kruger, & Wright, 2015). Weight concerns may be so severe that supplementary (particularly strength) training is avoided, as this is considered to alter body composition away from the dance ideal (Allen & Wyon, 2008). Moreover, evidence suggests greater eating psychopathology among dancers compared to non-dancers (for detailed review the reader is directed to Arcelus, Witcomb, and Mitchell (2014)), and dietary restraint and disordered eating have been reported in female tap, jazz, ballet and contemporary dancers (Robbeson et al., 2015), and mixed and contemporary dancers (Nordin-Bates, Walker, & Redding, 2011). When examining the prevalence of eating disorders among elite female athletes, Sundgot-Borgen (1993) determined that this was highest in aesthetic (34%) and weight dependent sports (27%), compared to endurance (20%), technical (13%) and ball-game sports (11%).

The expectation to maintain a lean form in dance has been evident for hundreds of years, and historically dancers have been characterised as sylph-like (Benson,

Gillien, Bourdet, & Loosli, 1985; Calabrese et al., 1983). Female ballet dancers in particular are reported to have low body weights, body mass index (BMI), and body fat levels (Calabrese et al., 1983; Cohen et al., 1985; Hamilton, Brooks-Gunn, Warren, & Hamilton, 1988; Laws, 2005; van Marken Lichtenbelt, Fogelholm, Ottenheijm, & Westerterp, 1995). However, this may not be the case for other genres, as ballerinas tend to be leanest (Pacy, Khalouha, & Koutedakis, 1996). Some studies have found body composition to be similar between ballet and contemporary dancers (Chmelar, 1988; White, Philpot, Green, & Bemben, 2004). However, more recently, a study which recruited a large cohort of trained dancers demonstrated that female ballet dancers had lower body mass (50.4 ± 4.4 vs 55.7 ± 6.3 kg), BMI (18.7 ± 1.3 vs 20.8 ± 1.8 $kg \cdot m^{-2}$), percentage body fat (%BF) (17.5 ± 2.5 vs 21.2 ± 3.8%) and were less muscular (3.4 ± 1.1 vs 4.1 ± 1.0 Mesomorphy Rating Scale) than contemporary counterparts (Liiv et al., 2013). Given the differences in physiological demands as well as discrete skills between contemporary and ballet dance genres (not least the gender roles in ballet requiring females to be lifted more frequently) (Wyon et al., 2011), this is perhaps unsurprising. Nevertheless, female contemporary dance students and graduates (Novak, Magill, & Schutte, 1978), and both intermediate and advanced contemporary dance students (Chatfield, Byrnes, Lally, & Rowe, 1990) have shown significantly lower %BF than non-dancers. Indeed, while much of the research has been concerned with ballet dancers, information regarding other dance styles suggests that they too desire to maintain a lean appearance (Clarkson, 1998).

2.1.2 Energy balance and energy availability

Energy balance (EB) is defined as total dietary energy intake (TEI) minus total energy expenditure (TEE); the summed expenditure from individual basal metabolic rate (BMR), the thermic effect of food (TEF; sometimes referred to as dietary induced thermogenesis), and exercise energy expenditure (EEE). A negative EB is indicative of weight loss, whereas a positive EB suggests weight gain. In contrast, energy availability (EA) is defined as TEI minus EEE; thus is described as the energy available for all other metabolic processes after consideration of physical activity (Loucks, Kiens, & Wright, 2011):

Energy balance = energy intake – total energy expenditure

Energy availability = energy intake – exercise energy expenditure

Typical EB in healthy female adults is achieved at an EA of approximately 45 kcal·kg fat free mass (FFM)$^{-1}$·day^{-1} and female dancers (as with other athletic females) are recommended to maintain an EA above 30 kcal·kg FFM^{-1}·day^{-1} to reduce the risk of disorders associated with energy imbalance (Sousa, Carvalho, Moreira, & Teixeira, 2013). For instance, long periods with low EA can impair not least physical performance, but also provoke medical complications involving (but not limited to) reproductive, skeletal, renal, cardiovascular, and central nervous systems (Nattiv et al., 2007). Specifically, potential issues arising from inadequate nutrition in dancers include insufficient peak bone mass and menstrual dysfunction (Kaufman et al., 2002; Warren, Brooks-Gunn, et al., 2002). Of concern, negative EB and a reduced stimulus for hypertrophy might reduce lean body mass, subsequently impair strength and performance, and increase susceptibility to injury (Carbone, McClung, & Pasiakos, 2012). Indeed, it has been suggested that very lean dancers are more prone to injury and importantly may take longer to recover than less lean counterparts (Benson, Geiger, Eiserman, & Wardlaw, 1989).

Despite this, studies regarding nutritional intake, energy expenditure, and dietary recommendations for dancers are scarce (Sousa et al., 2013). A recent review (Beck, Redding, & Wyon, 2015) has summarised the research investigating the energy demands of dance; largely through measurement of heart rate (HR) and volume of oxygen consumption ($\dot{V}O_2$). Though the authors conclude that the majority of investigations describe the energy demand to be moderate-high and intermittent, they noted a number of methodological limitations. Additionally, while these studies have identified energy demands in regards to a single movement, class, or performance, few have investigated these in nutritional contexts (i.e. kcal) or investigated the longer-term energy demands. While acute energy demands are important to understand, dancers regularly train in class, rehearsal, and have performances for several hours each day (please refer to section 2.2.4); thus it is important to quantify daily energy expenditure of dancers. In addition, in their resolve to achieve an aesthetic form, dancers are purported to consume low energy

intakes. Loucks (2004) reports that many athletes in aesthetic or weight dependent sports fail to compensate high energy demands with an adequate energy intake.

While many studies have sought to identify the dietary intakes of dancers, less have looked at this in parallel with their physical activity or energy expenditure (Beck, Mitchell, Foskett, Conlon, & von Hurst, 2015; Burckhardt, Wynn, Krieg, Bagutti, & Faouzi, 2011; Dahlstrom, Jansson, Nordevang, & Kaijser, 1990; Doyle-Lucas, Akers, & Davy, 2010; Frusztajer, Dhuper, Warren, Brooks-Gunn, & Fox, 1990; Hassapidou & Manstrantoni, 2001; Hirsch, Eisenmann, Moore, Winnail, & Stalder, 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002). Please refer to Table 1 for details regarding these publications. Consideration of energy expenditure is vital in determining the adequacy of energy intake in meeting the demands of dance, and enables EB and/or EA to be quantified. The majority of these studies determined that dancers were (for the most part) in negative EB or very low EA (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Doyle-Lucas et al., 2010; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002); with daily deficits ranging from approximately 549 kcal or 2.3 MJ (Beck, Mitchell, et al., 2015; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003) to 1319 kcal or 5.5 MJ (Warren, Brooks-Gunn, et al., 2002). However, these investigations used a range of measurement techniques to determine TEI and TEE, and whilst they provide an indication of EB, their validity has been questioned. It is important that the limitations associated with previous methods are acknowledged and taken into consideration when interpreting the literature. The following sections will discuss the methods used to examine energy intake and energy expenditure in dance populations in the aforementioned studies.

Table 1. Energy intake and energy expenditure in dancers

		Energy related measurement techniques		Reported energy status		
Author	**Subjects**	**Energy intake**	**Energy expenditure**	**Energy intake**	**Energy expenditure**	**Energy balance / availability**
Beck, Mitchell, et al., 2015	47 female adolescent ballet dancers (age 14.2 ± 1.2 y; BMI 19.7 ± 2.4 kg·m^{-2}; %BF 23.5 ± 4.1%). Self-reported training volume was 12.7 ± 6.1 h·week^{-1}.	4 day estimated food record (including 1 weekend day). Photographic portion guide and household measuring cups and spoons were provided. Analysed using the Foodworks Professional diet analysis program.	BMR was calculated using the Schofield equation. This was multiplied by a PAL of 1.8 for all dancers.	1935.3 ± 515.2 kcal·day^{-1} (or 8.1 ± 2.2 MJ·day^{-1}).	2485 ± 170 kcal·day^{-1} (or 10.4 ± 0.7 MJ·day^{-1}).	EB of -549 ± 345 kcal·day^{-1} (or -2.3 ± 1.4 MJ·day^{-1}).
Robbeson et al., 2015	26 female students (enrolled in Performing Arts programs) participating in African, tap, jazz, contemporary and ballet dancers (medians [25th; 75th percentiles] for age, BMI, and %BF were 19.0 y [18.0; 21.0], 21.2 kg·m^{-2} [19.9; 22.2], and 22.8% [19.4; 27.9] respectively).	5 day weighed food record (including training and non-training days). Dietary data were coded using the Medical Research Council Condensed Food Composition Tables for South Africa, captured in an Excel spreadsheet, and converted to energy. Completed during training and auditioning periods.	During the same 5 days the food record was kept, an Actiheart monitor was worn. This estimated EE by summing resting metabolic rate (Schofield equation), activity energy expenditure and DIT.	Median (25th; 75th percentiles) was 1849 (1576; 2368) kcal·day^{-1} (or 7.7 (6.6; 9.9) MJ·day^{-1}).	Median (25th; 75th percentiles) was 2832 (2201; 3129) kcal·day^{-1} (or 11.9 (9.2; 13.1) MJ·day^{-1}).	Median (25th; 75th percentiles) EB was -931 (-1251; -292) kcal·day^{-1} (or -3.9 (-5.2; -1.2) MJ·day^{-1}) and EA was 39 (30; 46) kcal·kg FFM^{-1}·day^{-1}. 24% of dancers had EA <30 kcal·kg FFM^{-1}·day^{-1}
Hoch et al., 2011	22 elite female ballet dancers (age 23.2 ± 4.7 y; BMI 19.3 ± 1.1 kg·m^{-2}; %BF 16.7 ± 4.9%). Self-reported 17.0 ± 4.5 y of elite dancing.	3 day food record (including 1 weekend day) during the training season. Participants were encouraged to weigh their food. The Nutrition Data System for Research software was used to analyse the diet.	Participants wore an accelerometer for 3 days to determine exercise energy expenditure.	Not reported	Not reported	The authors reported that seventeen dancers (77%) had evidence of low/negative energy availability when calculated by subtracting exercise EE from EI (−547.8 ± 359.9 kcal·day^{-1} or 2.3 ± 1.5 MJ·day^{-1}). However, given that BMR, TEF, and kg FFM were not measured, this cannot be confirmed.

Table 1. continued

		Energy related measurement techniques		Reported energy status		
Author	**Subjects**	**Energy intake**	**Energy expenditure**	**Energy intake**	**Energy expenditure**	**Energy balance / availability**
Burckhardt et al., 2011	127 female adolescent ballet dancers (age 16.7 ± 0.8 y; BMI 17.8 ± 1.3 $kg \cdot m^{-2}$). Conducted during pre-professional dance competition.	A 3 day qualitative dietary record was completed (including 1 weekend day). The approximate quantity of foods consumed was noted as portions.	Self-reported dance activity.	Participants were not required to weigh food, so no data on total energy and macronutrient intake were available. Food intake, evaluated by number of consumed food portions, was below the recommendations for a normally active population in all food groups except animal proteins.	The reported hours of dance activity (22.1 ± 7.6 $h \cdot week^{-1}$). were suggested to correspond to high-intensity activity, equivalent to 5100 $kcal \cdot week^{-1}$ (or 21.3 $MJ \cdot week^{-1}$). However, this was not directly measured.	Not reported
Doyle-Lucas et al., 2010	15 female elite ballet dancers (age 24.3 ± 1.3 y; BMI 18.9 ± 0.2 $kg \cdot m^{-2}$; %BF 15.5 ± 1.3%) from two national professional companies. More than 27 $h \cdot week^{-1}$ of dancing.	4 day food records. Measuring spoons, cups and food models were used to determine portion sizes. These were analysed using the Nutritional Data System for Research nutritional analysis software program.	RMR was assessed by indirect calorimetry. Using self-reported data, exercise energy expenditure was calculated from the equation METs x $kcal \cdot h^{-1}$ (from RMR) x h exercising.	1577 ± 89 $kcal \cdot day^{-1}$ (or 6.6 ± 0.4 $MJ \cdot day^{-1}$).	Dancers reported habitual moderate-high intensity physical activity levels of approximately 36 $h \cdot week^{-1}$. Absolute values for EE were not reported.	EA of dancers was reported to be 3.75 ± 2.2 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ and as low as 0.6 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ in those with irregular menses and amenorrheic dancers.

Table 1. continued

		Energy related measurement techniques		Reported energy status		
Author	**Subjects**	**Energy intake**	**Energy expenditure**	**Energy intake**	**Energy expenditure**	**Energy balance / availability**
Kostrzewa-Tarnowska & Jeszka, 2003	44 adolescent female ballet dancers (age 13 ± 1 y; 17.1 ± 1.6 $kg \cdot m^{-2}$; BF 14.5 ± 4.1%) from a professional elite Dance School were recruited.	24 h dietary recall interview. The Photographic Album of Dishes was used and EI was calculated based on food composition tables with the application of Dietetyk computer program.	24 h heart rate monitoring was used to determine resting and activity EE (FLEX HR was used: individual relationship between HR and $\dot{V}O_2$ was established). EE during sleep was assumed to be equal to BMR (Schofield equation).	2075 ± 538 $kcal \cdot day^{-1}$ (or 8.68 ± 2.25 $MJ \cdot day^{-1}$).	2639 ± 311 $kcal \cdot day^{-1}$ (or 11.04 ± 1.3 $MJ \cdot day^{-1}$).	EB of the dancers was -562 ± 511 $kcal \cdot day^{-1}$ (or -2.35 ± 2.14 $MJ \cdot day^{-1}$).
Hirsch et al., 2003	Three male (age 22.3 ± 1.2 y; BMI 21.8 ± 3.6 $kg \cdot m^{-2}$; %BF 9.4 ± 2.8%) and 11 female (age 20.2 ± 1.1 y, BMI 21.4 ± 1.9 $kg \cdot m^{-2}$; %BF 17.2 ± 3.0%) university level ballet dancers. Minimum of 15 $h \cdot week^{-1}$ of dancing.	3 day food record (including 1 weekend day) during training and rehearsal periods. Food records were entered into a Nutrition Analysis Tool.	3 day heart rate monitoring was used to determine EE (FLEX HR was used: individual relationship between HR and $\dot{V}O_2$ was established). EE during sleep was assumed to be equal to BMR (Schofield equation).	2755 ± 847 and 2080 ± 371 $kcal \cdot day^{-1}$ (or 11.5 ± 3.5 and 8.7 ± 1.5 $MJ \cdot day^{-1}$) for male and female dancers respectively.	4617 ± 1244 and 2945 ± 823 $kcal \cdot day^{-1}$ (or 19.3 ± 5.2 and 12.3 ± 3.4 $MJ \cdot day^{-1}$) for male and female dancers respectively.	EB was -1862 and -865 $kcal \cdot day^{-1}$ (or 7.6 and 3.6 $MJ \cdot day^{-1}$) for male and female dancers respectively.
Warren, Brooks-Gunn, et al., 2002	54 female ballet dancers, 22 of whom were amenorrheic (age 19.2 ± 3.4 y; %BF 20.9 ± 3.9%) and 32 eumenorrheic (age 22.0 ± 4.7 y; %BF 23.3 ± 3.4%).	Food intake was determined using a 2 day dietary recall (two 24 recall diaries) and a FFQ. These were coded using the Nutri-calc software package.	3 day self-reported activity questionnaire (including 1 weekend day). Activity level was determined based on the number of calories expended per day according to the method of Bouchard et al. (1983).	1620.2 ± 597.9 and 1765.1 ± 642.9 $kcal \cdot day^{-1}$ (or 6.8 ± 2.5 and 7.4 ± 2.7 $MJ \cdot day^{-1}$) for eumenorrhoeic and amenorrhoeic dancers respectively.	2939.0 ± 590.0 and 2674.9 ± 614.4 $kcal \cdot day^{-1}$ (or 12.3 ± 2.5 and 11.2 ± 2.6 $MJ \cdot day^{-1}$) for eumenorrhoeic and amenorrhoeic dancers respectively.	EB was -1318.8 and -909.8 $kcal \cdot day^{-1}$ (or -5.5 and -3.8 $MJ \cdot day^{-1}$) for eumenorrhoeic and amenorrhoeic dancers respectively.

Table 1. continued

		Energy related measurement techniques		Reported energy status		
Author	**Subjects**	**Energy intake**	**Energy expenditure**	**Energy intake**	**Energy expenditure**	**Energy balance / availability**
Hassapidou & Manstrantoni, 2001	35 female athletes (18 ± 26 y) including eight volleyball players, 11 middle distance runners, nine swimmers and seven ballet dancers (BMI 20.1 ± 1.3 $kg \cdot m^{-2}$; %BF 18.6 ± 2.5% in training and BMI 19.7 ± 1.3 $kg \cdot m^{-2}$; %BF 17.8 ± 3.6% in competition).	Data were collected over two seasons, the training and the competitive. A 7 day weighed dietary record was used to estimate EI. Dietary intakes were analysed using the Microdiet computer program.	Data were collected over two seasons, the training and the competitive. A 7 day activity record was used to estimate EE. All activities were converted to METs equivalents. RMR was estimated (Owen equation).	1701 ± 580 and 1506 ± 468 $kcal \cdot day^{-1}$ (or 7.1 ± 2.4 and 6.3 ± 2.0 $MJ \cdot day^{-1}$) in training and competition respectively.	2344 ± 126 and 2221 ± 254 $kcal \cdot day^{-1}$ (or 9.8 ± 0.5 and 9.3 ± 1.1 $MJ \cdot day^{-1}$) in training and competition respectively.	EB of approximately -643 and -715 $kcal \cdot day^{-1}$ (or -2.7 and -3.0 $MJ \cdot day^{-1}$) in training and competition respectively.
Dahlstrom et al., 1990	14 female dance students (age 23.7 ± 2.1 y; BMI 20.7 ± 1.7 $kg \cdot m^{-2}$). Genre of dance was not specified.	Food habits and EI were analysed using a dietary history method (retrospective interview method). The Swedish National Food Adminstration files were consulted to estimate nutrient and energy content of food.	Two different methods of calculation were used. 1) the energy requirement of the dancers was estimated by the formula recommended by WHO. 2) the rate of expenditure during training sessions was added to the WHO formula.	1984 ± 454 $kcal \cdot day^{-1}$ (or 8.3 ± 1.9 $MJ \cdot day^{-1}$).	2457 ± 157 and 2976 ± 421 $kcal \cdot day^{-1}$ (or 10.3 ± 0.7 and 12.6 ± 1.8 $MJ \cdot day^{-1}$) for method 1 and 2 respectively.	EB of approximately -478 $kcal \cdot day^{-1}$ (or -2 $MJ \cdot day^{-1}$).

Table 1. continued

		Energy related measurement techniques		Reported energy status		
Author	**Subjects**	**Energy intake**	**Energy expenditure**	**Energy intake**	**Energy expenditure**	**Energy balance / availability**
Frusztajer et al., 1990	20 female ballet dancers (of 45 recruited); 10 dancers who experienced a stress fracture in last year (20.5 ± 3.9 y) and 10 who had not had a stress fracture in last 3 years (20.5 ± 4.0 y).	Food intake determined by 2 day dietary history (two 24 h recall diaries) and a FFQ. These were coded using the Nutri-calc software package.	3 day self-reported questionnaire (including 1 weekend day). Activity level was determined based on the number of calories expended per day according to the method of Bouchard et al. (1983).	According to FFQ, EI for those who experienced a stress fracture in the last year and those who did not was 1139.1 ± 264.7 and 1431.6 ± 500.2 kcal·day^{-1} (or 4.8 ± 1.1 and 6.0 ± 2.1 MJ·day^{-1}) respectively. According to 2 day dietary history, EI for those who experienced a stress fracture in the last year and those who did not was 1482.5 ± 523.5 and 1691.6 ± 505.1 kcal·day^{-1} (or 6.2 ± 2.2 and 7.1 ± 2.1 MJ·day^{-1}) respectively.	Not reported.	Not determined.

BMI, body mass index; %BF, percentage body fat; EI, energy intake; EE, energy expenditure; EB, energy balance; EA, energy availability; PAL, physical activity level; DIT, dietary induced thermogenesis; TEF, thermic effect of food; FFQ, food frequency questionnaire; METs, metabolic equivalents; WHO, World Health Organisation; BMR, basal metabolic rate; RMR, resting metabolic rate; FFM, fat free mass; HR, heart rate; $\dot{V}O_2$, volume of oxygen consumption.

2.1.3 Measuring energy intake

A gold-standard method of assessing energy intake is considered to be the observation technique (Baker, Heaton, Stein, Nuccio, & Jeukendrup, 2014). This requires an investigator to observe and precisely record the *ad libitum* consumption of pre-prepared food and drink consumed by an individual, typically in a confined environment. Therefore, this method is resource intensive and is not conducive to free-living eating behaviour. The observation technique has not been used to assess EB in dance populations, rather research has employed methods including self-report food records (in some cases weighed), 24-hour recall interviews, and the use of dietary history questionnaires such as food frequency questionnaires (FFQs). These allow a measure of habitual energy intake to be determined from free-living conditions and therefore are more representative of true eating behaviour.

Self-report food records have been used in many studies investigating energy intake and energy expenditure in dancers (Beck, Mitchell, et al., 2015; Burckhardt et al., 2011; Doyle-Lucas et al., 2010; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Robbeson et al., 2015). This method is considered a prospective technique since it usually requires food intake to be recorded at the time of consumption (Ashley & Bovee, 2007). Self-report food diaries are typically collected for 3 to 7 consecutive days, however a 7-day period is thought to best represent a variety of dietary practices and is associated with the most valid nutritional information (Bingham, 1987; Black et al., 1991). Interestingly, only one study has used a 7-day period to identify energy intake with weighed food records in female ballet dancers (among other female athletes) (Hassapidou & Manstrantoni, 2001), the remainder have used periods of 5 days or fewer. There are a number of inherent limitations associated with self-report food record, largely arising from the required motivation and commitment from the participants themselves. Certainly, for accurate interpretation, individuals are required to record a great deal of information; not least regarding the food consumed, but also brand names, recipes, preparation and cooking methods, and quantities consumed via household measures or using weighing scales. It is the responsibility of the individual alone to record all intakes and therefore the burden and tedium associated with recording food items on

a frequent basis, forgetfulness, and overall lack of compliance are errors that limit this assessment technique.

Additional reasons for low energy intakes reported by dietary records in dance populations have been suggested to include deliberate under-reporting and/or under-eating due to the desire for weight loss (Dahlstrom et al., 1990; Robbeson et al., 2015). As observed in many populations, Hassapidou and Manstrantoni (2001) suggested that not only is it possible that dancers fail to record portions of food correctly, but they may also omit foods eaten or restrict their food intake during the study period. A number of investigations (Crawley & Summerbell, 1997; Heitmann, 1993; Lafay et al., 1997) have identified that individuals might under-report energy intake as an artefact of dietary restraint (a tendency to consciously control food intake in order to assist weight loss or prevent weight gain), and that in females, restrained eaters under-report to a greater extent than unrestrained eaters (Bathalon et al., 2000). Interestingly, evidence suggests that under-reporting may be unconscious and associated with perceived body image and body dissatisfaction (Edwards, Lindeman, Mikesky, & Stager, 1993). Given the previously described prevalence of restrained and disordered eating practices in dance populations (section 2.1.2), these limitations should be considered when interpreting literature using such methods in dancers. Indeed, a study in female ballet dancers reported a mean bias to under-reporting of 667 $kcal \cdot day^{-1}$ or 21% of energy intake when comparing four-day weighed food recording and energy expenditure via doubly labelled water (DLW; discussed in section 2.1.4) (Hill & Davies, 1999).

An alternative method that has been used in studies assessing energy intake in dancers is the 24-hour recall interview technique (Frusztajer et al., 1990; Kostrzewa-Tarnowska & Jeszka, 2003; Warren, Brooks-Gunn, et al., 2002). In contrast to the prospective method of self-report food records, 24-hour recall interview is retrospective as information regarding previous food intake is collected (Ashley & Bovee, 2007). The interview is typically conducted by a trained individual (Johnson, 2002), using the two-pass or multiple-pass method; whereby initial information provided by the individual is reviewed and participants are prompted for further details which may otherwise have been overlooked (Ashley & Bovee, 2007). As a result, this method requires less participant burden compared to a food record, is easy, cheap and relatively quick to administer, and when administered unannounced

it is unlikely that dietary habits are misrepresented by the individual (Johnson, 2002). However, due to its retrospective nature, it relies on the participant's ability to recall foods consumed and their ability to estimate portion sizes. The credibility of recall interview methods was previously questioned by Kostrzewa-Tarnowska and Jeszka (2003) given that their results demonstrated a large imbalance between TEI and TEE in female ballet dancers. However, in a recent study, 24-hour recall (multiple-pass method) was found to have strong agreement with the gold-standard observation technique in a large population of competitive athletes, including jazz, ballet and modern dancers (Baker et al., 2014).

Another retrospective technique to determine energy intake is the use of questionnaires regarding dietary history, and these have been used by a small number of investigations in dancers (Dahlstrom et al., 1990; Frusztajer et al., 1990; Warren, Brooks-Gunn, et al., 2002). Dahlstrom et al. (1990) used questionnaires in an interview format based on previous methods (Isaksson, 1980). Frusztajer et al. (1990) and Warren, Brooks-Gunn, et al. (2002) used a semi-quantitative FFQ (Willett et al., 1985) which was used in combination with 24-hour recall interviews. Reproducibility and validity of this questionnaire were quantified in a large sample of females; and mean intraclass correlation coefficient comparing intakes of 18 nutrients measured using the questionnaire and a 7-day dietary recall method was 0.60 (Willett et al., 1985). While FFQs require minimal training and are easy to administer, they attempt to assess habitual energy intake usually over the past 12 months, and thus rely heavily on long-term memory (Adamson et al., 2009). Indeed, females completing FFQs have been shown to under-report energy intake by approximately 10% compared to DLW (Andersen, Tomten, Haggarty, Lovo, & Hustvedt, 2003).

Accuracy of estimates of energy intake are thought to be enhanced when methods of assessment are used in combination compared to individual methods alone (Shim, Oh, & Kim, 2014). Certainly, using a second method can substantiate information provided by the first, whilst also helping to address and minimise measurement errors associated with the individual techniques used in isolation. For instance, in adolescent athletic populations, using self-report weighed food diary in conjunction with 24-hour recall interview has been found to result in good agreement with the

gold-standard observed food intake technique (Briggs, Rumbold, Cockburn, Russell, & Stevenson, 2015; Rumbold, St Clair Gibson, Stevenson, & Dodd-Reynolds, 2011). No studies have compared the combined self-report food diary and 24-hour recall interview technique to observation techniques, nor to DLW as a reference to determine the validity of its use in dance populations. Nonetheless, these data suggest a potential application of this combined method approach to assess TEI in female dancers.

2.1.4 Measuring energy expenditure

Compared to energy intake, energy expenditure is arguably more difficult to estimate, as it typically requires cumbersome and less affordable techniques to obtain accurate measurements (McMinn, Acharya, Rowe, Gray, & Allan, 2013). As previously described (section 2.1.2), TEE is comprised of BMR, TEF, and EEE (Leenders, Sherman, Nagaraja, & Kien, 2001) and therefore each of these contributing elements must be considered in order to accurately assess TEE. The methods used to measure TEE are discussed in this section, with specific reference to dance populations.

The DLW method is considered the gold-standard for measuring long-term averages of TEE in field conditions (Shephard & Aoyagi, 2012). A known dose of DLW containing stable non-radioactive isotopes (usually ^{18}Oxygen and ^{2}Hydrogen) is administered, and following equilibration with body fluids, the rates of elimination of these isotopes are determined from samples of blood, saliva or urine typically over an interval of two weeks (Shephard & Aoyagi, 2012). The advantage of this method is that it is simple and non-invasive, and between collection of initial and final samples, allows participants to continue normal eating and exercise behaviours. Therefore, it is an accurate measurement of TEE in truly free-living individuals (Levine, 2005). However, this method is costly (in time, materials and isotope analysis), and not without its limitations; namely that it does not offer information regarding the nature of exercise performed (intensity, frequency, duration), nor does it measure the TEF (Levine, 2005). Therefore, it is used primarily to validate other methods of estimating TEE. This method has been used in one dance cohort, which

examined the validity of using four-day weighed food record compared to the DLW technique in female ballet dancers (Hill & Davies, 1999).

Individual BMR accounts for the largest proportion (approximately 60%) of TEE (Levine, 2005), and therefore warrants careful assessment. Most accurate and precise measurements of BMR (and indeed all components of TEE) are conducted through measurement of heat loss using direct or indirect calorimetry (Levine, 2005). Despite this, few laboratories have access to the required resources for direct calorimetry (metabolic whole-body chambers and technical expertise), measurement periods are lengthy, and given it confines participants, this method cannot be used in field and free-living situations (Shephard & Aoyagi, 2012). Similarly, indirect calorimetry (namely using open-circuit systems and respiratory chambers) requires participants to be confined in artificial and restricted conditions, likely implicating behaviour change. Though relatively light-weight portable devices have been developed for use in the field (Levine, 2005), these devices nevertheless remain obtrusive. Indeed, a major limitation of most respiratory devices is the need to use a mouthpiece and nose clip, or a facemask (Shephard & Aoyagi, 2012). One study has used indirect calorimetry for determination of resting metabolic rate (RMR) in female ballet dancers using a ventilated hood system (measured over 45 mins at rest, following a 10-12 h fast), which was then added to estimated EEE to determine TEE (Doyle-Lucas et al., 2010). Given the obtrusive nature of direct and indirect calorimetry, it is not feasible to employ these methods for assessment of average TEE over a prolonged period.

Consequently, the majority of investigations assessing EB in dancers have sought to identify BMR using predictive equations, typically considering body mass, stature, sex and age (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015). The Harris-Benedict equation (Harris & Benedict, 1918) validated elsewhere (Roza & Shizgal, 1984) is the most widely used predictive equation (Frankenfield, Muth, & Rowe, 1998). Interestingly, compared to the Cunningham (Cunningham, 1980) and Mifflin (Mifflin et al., 1990) equations, the Harris-Benedict best predicted BMR in female dancers compared to observed values using indirect calorimetry (Doyle-Lucas et al., 2010). It is important to note that evidence suggests dancers (as with other athletic females), may be more

metabolically efficient compared to controls relative to FFM, potentially due to the suppression of BMR with low energy intake (Doyle-Lucas et al., 2010; Kaufman et al., 2002). However, others have reported no differences in RMR nor TEF between amenorrheic and eumenorrheic ballet dancers (Glace, Kremenic, & Liederbach, 2006). Therefore, these authors concluded that discrepancies between energy intakes and expenditures are perhaps due to misreporting rather than mechanisms of energy conservation. Moreover, the inflammatory response (Jamurtas et al., 2004) and the repair of damaged muscle following strenuous exercise is reported to increase energy expenditure; with an estimated 20% of RMR explained by the energy demand of protein turnover (Welle & Nair, 1990). In a recent study (Burt, Lamb, Nicholas, & Twist, 2014) it was demonstrated that RMR increased by up to 13.2%, with a concomitant increase in $\dot{V}O_2$ during exercise in the subsequent days following exercise-induced muscle damage (EIMD). Therefore, researchers investigating populations who may be exposed to muscle-damaging exercise during free-living conditions, should be mindful of these increases in resting and exercise energy expenditure.

The TEF is defined as the increase in metabolic rate after ingestion of a meal, and in a mixed diet can account for approximately 10% of TEE (Reed & Hill, 1996). However, TEF varies among macronutrients; that of lipids, carbohydrate, and protein equates to 2-3, 6-8, and 25-30% of their intake, respectively (Jequier, 2002). Despite its role in TEE, only one study investigating EB in dancers has acknowledged the measurement of TEF, which was calculated as a constant 10% of TEE using the Actiheart (CamNtech Ltd, Cambridge, UK) accelerometer (Robbeson et al., 2015). The lack of inclusion of TEF in the estimation of TEE represents a major limitation in this area of research as intuitively, TEE may be underestimated in the order of approximately 10%.

Energy expended through physical activity, is the most variable component of TEE (Westerterp, 2015), and a variety of methods have been used to determine EEE in dance populations. Some research studies have relied upon self-reported logs to assess EEE; specifically, by applying Metabolic Equivalents of Task (MET) to reported activities (Doyle-Lucas et al., 2010; Frusztajer et al., 1990; Hassapidou & Manstrantoni, 2001; Warren, Brooks-Gunn, et al., 2002). The detail recorded in

activity diaries and logs vary and they have a high administrative burden (Ainsworth, 2009), which challenges participant motivation and compliance. In addition, physical activity is often over-reported, creating systematic and random errors similar to those observed in self-reported EI (Dhurandhar et al., 2015). Others have multiplied estimated BMR by a constant physical activity level to determine TEE (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990). This method assumes the same activity level for all participants and should not be used when research is interested in precise measurement of EEE.

In humans, there is a significant relationship between HR and energy expenditure (Levine, 2005) and HR monitoring is the most commonly employed method for quantifying work intensity during dance performance (Domene & Easton, 2014). A number of investigations examining EB in dancers have used this technique (Dahlstrom et al., 1990; Hirsch et al., 2003; Kostrzewa-Tarnowska & Jeszka, 2003). Typically, this method requires participants to wear an elastic strap around the chest which transmits HR data by telemetry to a small wrist-watch-type receiver. These monitors are portable, unobtrusive, and non-restraining, and are capable of carrying out measurements over several days (Levine, 2005). However, it is well documented that there are a number of co-variables that affect HR including emotion, environment, hydration and nutritional status, fitness level, body composition, illness, cardiac stroke volume, haemoglobin content, and blood flow (Levine, 2005; Li, Deurenberg, & Hautvast, 1993). Scharff-Olson, Williford, and Smith (1992) also suggested that the excitatory effect of music can have implications on HR in dance. Evidently, there is high inter-individual variability of the HR and energy expenditure relationship and differentiation between increases in HR as a result of exercise or as a result of the aforementioned co-variables is not possible with HR monitors.

In an attempt to address inter-individual differences, researchers are advised to create individual regression equations for each participant to enhance the precision of HR prediction (Levine, 2005). This method was adopted in two studies examining energy intake and energy expenditure of male and female ballet dancers (Hirsch et al., 2003; Kostrzewa-Tarnowska & Jeszka, 2003). Each participant wore a HR monitor and a portable gas analyser in various resting activities (lying supine,

sitting quietly and standing quietly) and at various speeds during a continuous, progressive cycle ergometer and/or treadmill test in order to calculate individual HR-$\dot{V}O_2$ regression equations. These authors then adopted the FLEX HR technique developed by Spurr et al. (1988) to assess energy expenditure. FLEX HR was calculated as the mean of the highest HR for the resting activities and the lowest HR of the exercise activities (Ceesay et al., 1989), and was used as a threshold to classify sedentary (HR below FLEX HR) and exercise activity (HR above FLEX HR) during a three-day period of HR monitoring. EEE was then evaluated according to the individual participants' pre-determined HR-$\dot{V}O_2$ linear regression. This method provides a temporal representation of the intensity, frequency and daily variation of exercise performed (Spurr et al., 1988) and does not typically induce changes in behaviour. In addition, the FLEX HR method has been validated against DLW in adults (Livingstone et al., 1990). However, measurements of estimated energy expenditure in the aforementioned studies were based on the assumption that the HR-$\dot{V}O_2$ relationship established during cycle ergometer and/or treadmill exercise was the same as the relationship during dance activity. For accurate estimation of energy expenditure, the activities used in the calibrations should be representative of the activities likely to be monitored (Ceesay et al., 1989). Indeed, HR has been shown to increase during aerobic dance compared to treadmill exercise at the same $\dot{V}O_2$ (Scharff-Olson et al., 1992). In addition, a study determined differences between the HR-$\dot{V}O_2$ relationship during a multi-stage graded treadmill test and during a contemporary dance class at lower intensities (though appeared to compare at higher intensities) (Redding, Wyon, Shearman, & Doggart, 2004). The authors suggested that given its intermittent nature, HR is not reliable indicator for estimations of $\dot{V}O_2$ (and therefore energy expenditure) in contemporary dance.

Finally, more recently, accelerometers have been used to determine energy expenditure in studies assessing EB in dance populations (Hoch et al., 2011; Robbeson et al., 2015). Use of accelerometers has been recommended given their usability, relatively low cost, and the information they provide regarding the intensity, duration and frequency of physical activity (Leonard, 2012). These electronic devices measure daily activity and energy expenditure objectively, and in real time by assessing the body's movement and acceleration on single or multiple orthogonal planes. Specifically, uni-axial accelerometers are sensitive to

acceleration in the vertical plane, omni-directional accelerometers also consider the medio-lateral plane, and tri-axial accelerometers use additional measures in the anterior-posterior directions (thought to be more precise than uni-axial devices (Levine, 2005)). Accelerometers are typically worn around the waist, chest, ankle or wrist, and inbuilt mathematical algorithms are used to determine energy expenditure from the raw acceleration measured on the axis or axes. Therefore, the energy expenditure estimations are dependent on the algorithm applied, rather than the raw data generated by the device (McMinn et al., 2013). Typically, these prediction models are generated from simple exercise tasks such as walking and running in laboratory conditions, and investigation of the devices during more complex free-living activities is warranted, for instance during household chores, gardening and more multifaceted exercise modes. Indeed, a major limitation in using accelerometry is in applying these predication models across a range of modes and intensities of activity. One study (Domene & Easton, 2014) has generated value calibrations for the determination of energy expenditure, step count, and the development of physical activity intensity cut-points specific to Latin dance, however models that are specific to other styles of dance are yet to be established.

ActiGraphs are the most validated of commercially available accelerometers used in physical activity research (Plasqui, Bonomi, & Westerterp, 2013). Hoch et al. (2011) used a uni-axial ActiGraph (Actigraph GT1M, Pensacola, Florida, USA) to estimate EEE in female ballet dancers, however, these authors failed to identify which algorithm was used to estimate energy expenditure. Moreover, the location of the accelerometer was not reported, and this too has implications on estimating energy expenditure (Bouten, Sauren, Verduin, & Janssen, 1997). Indeed, a recent study determined that the most recent tri-axial Actigraph (Actigraph GT3X+, Pensacola, Florida, USA) worn on the wrist (compared to ankle or hip mounted) with simultaneous HR monitoring best predicted energy expenditure during Latin dance performance when compared to indirect calorimetry (Domene & Easton, 2014). This may be explained by the fact that there is a substantial contribution from upper body movements, particularly the upper torso and arms, during Latin dance performance (Domene & Easton, 2014). However, given that the physical and technical demands of dance are dependent on the genre, wrist mounted accelerometers might not be applicable for other dance styles. In addition, when estimating energy expenditure,

the Actigraph GT3X+ has demonstrated superior correlation with indirect calorimetry when worn on the waist (r = 0.82) compared to on the wrist (r = 0.72), and step count estimates were accurate across a continuum of exercise intensities when waist but not wrist mounted compared to researcher observed step counts (McMinn et al., 2013). Finally, the most recent prediction equation generated for adult populations using the Actigraph GT3X+ (Sasaki, John, & Freedson, 2011) was developed using data from waist-worn devices. Therefore, researchers should be cautious regarding positioning the device elsewhere when using this algorithm to estimate EEE.

While accelerometers yield precise data from standardised laboratory activities, in free-living conditions they remain vulnerable to limitations associated with complex movement tasks, external vibration, and a failure to measure sources of energy expenditure such as hill climbing or against external resistance (Shephard & Aoyagi, 2012). In addition, developing accurate algorithms to estimate energy expenditure for a variety of activities remains a challenge (Leonard, 2012). Despite its limitations, without the development of activity specific models or more advanced pattern recognition techniques, using inbuilt mathematical algorithms remains the method of choice to classify physical activity and energy expenditure with accelerometers (Sasaki et al., 2011). Moreover, these devices offer a practical, relatively low cost, and unobtrusive means of estimating EEE continually over prolonged periods of time.

2.1.5 Summary of energy balance in dance

Maintaining a lean physique is thought to be an important aspect of dance fitness and a pre-requisite for success in the profession (Claessens et al., 1987; Hergenroeder et al., 1993). Consequently, as with many athletes in aesthetic or weight dependent sports (Loucks, 2004), dancers might fail to compensate high energy demands with an adequate energy intake, and are at risk of numerous health and performance impairments associated with energy imbalance. Despite this, little is known about the energy intakes and energy expenditures of female dancers.

Accurate determination of EB requires careful measurement of all its components; TEI and TEE comprised of BMR, TEF, and EEE. Measurement techniques of TEI and TEE should be considered with regard to the specific population, the purpose, and the constraints (cost, logistics, time and resources) associated with a research study to determine the most appropriate methods. Collecting reliable and accurate information regarding nutrition in dance populations is challenging, and it is recognised that methods used to date (namely self-reported dietary recording) are limited by under/over eating and/or reporting (Magkos & Yannakoulia, 2003). Interestingly, it appears that the use of combined methods may be appropriate for more accurate estimation of EI in free-living conditions, and should be considered in future research. Determination of TEE is equally important, in order to provide a greater understanding regarding the energy demands placed on dancers, and whether these are being met by TEI. Accurate assessment of TEE is difficult in free-living conditions, as many measurement techniques are costly, obtrusive and/or restrictive, implicate behaviour change, or interfere with daily living. Practical measurement techniques that are capable of estimating TEE in free-living conditions over a number of days should be considered when assessing EB in dance populations in future research.

Although the low BMI and body fat levels frequently reported in dancers (Calabrese et al., 1983; Cohen et al., 1985; Hamilton et al., 1988; Laws, 2005; van Marken Lichtenbelt et al., 1995) suggest that exercise and/or eating behaviours may be suboptimal, the inherent limitations in study designs render previous conclusions of poor nutritional intake and EB questionable. Moreover, while one study has been conducted in South African dancers from a variety of genres (including contemporary) (Robbeson et al., 2015), the remainder have recruited female and/or male ballet dancers. No study has looked to identify the energy intakes and energy expenditures of modern/contemporary equivalents alone. Given the differences in artistic and physical demands (Wyon et al., 2011), and differences in body composition (Liiv et al., 2013) between ballet and contemporary genres, this is an important limitation in the literature which has yet to be addressed.

2.2 Exercise-induced muscle damage

2.2.1 Introduction

Many exercise activities can result in exercise-induced muscle damage (EIMD), however novel and eccentric-biased activity results in greater levels of EIMD compared to other muscle actions (Gibala et al., 2000; Penailillo, Blazevich, Numazawa, & Nosaka, 2013). It has long been recognised that the energy requirements of eccentric (muscle lengthening) contractions are lower than concentric (muscle shortening) contractions (Knuttgen, Petersen, & Klausen, 1971) and that eccentric contractions produce significantly greater forces (as evidenced by the force-velocity relationship (Fenn & Marsh, 1935)). However, eccentric contractions recruit fewer fibres compared to concentric contractions (Enoka, 1996), and consequently, for the same power output and at a lower energy cost, can cause greater muscle damage attributed to a higher load and mechanical stress per fibre (Newham, Mills, Quigley, & Edwards, 1983). This damage appears greater still when the velocity of eccentric contractions increases (Chapman, Newton, McGuigan, & Nosaka, 2008).

The EIMD phenomenon manifests as an increase in muscle soreness and swelling, increased appearance of systemic indices associated with EIMD, and a decrease in muscle functionality (Howatson & van Someren, 2008) (discussed in detail in section 2.2.2). Though temporary, these negative symptoms of EIMD can be relatively long-lasting, and importantly have the potential to interfere with the training and performance demands of athletic populations. While well-researched, the precise mechanisms of EIMD are not wholly understood; however the aetiology is thought to be a bi-phasic process and often divided into a two-part model. This describes a *primary* response involving a combination of mechanical and metabolic effects precipitated during the exercise bout, and a *secondary* response characterised by an increase in inflammation; exacerbating and prolonging the initial damage (Howatson & van Someren, 2008). The proposed mechanisms of EIMD will be discussed in this section, as well as methods to assess EIMD, and the determinants which might influence the severity of damage. This is then followed by a review of the evidence regarding muscle damage in dance.

2.2.1.1 Primary response

Initial muscle damage is proposed to manifest as a direct result of high mechanical tension on the myofibril during contraction (Proske & Morgan, 2001). During eccentric contractions the muscle lengthens in a non-uniform manner whereby adjacent sarcomeres in parallel undergo differing degrees of lengthening. As a result, some sarcomeres may be 'overstretched' beyond myofilament overlap. The lack of interaction between the active structures (actin and myosin) results in the transfer of tension to passive structures such as desmin and titin (Howatson & van Someren, 2008), subsequently causing sarcomere 'popping' (Morgan, 1990). Sarcomeres that have been overstretched may be unable to re-interdigitate for subsequent contraction, which places neighbouring sarcomeres under greater tension; ultimately causing further disruption. Consequently, a cumulative effect of repeated contractions might result in large areas of disrupted sarcomeres, which can eventually lead to tearing of membranes, including the sarcolemma, transverse (t) tubules, or sarcoplasmic reticulum (SR) (Morgan & Allen, 1999). Histological alterations provide direct evidence of this mechanical muscle damage (Figure 1) and the compromised structure of muscle fibres is likely to impair the muscle's ability to generate force (Howatson & van Someren, 2008).

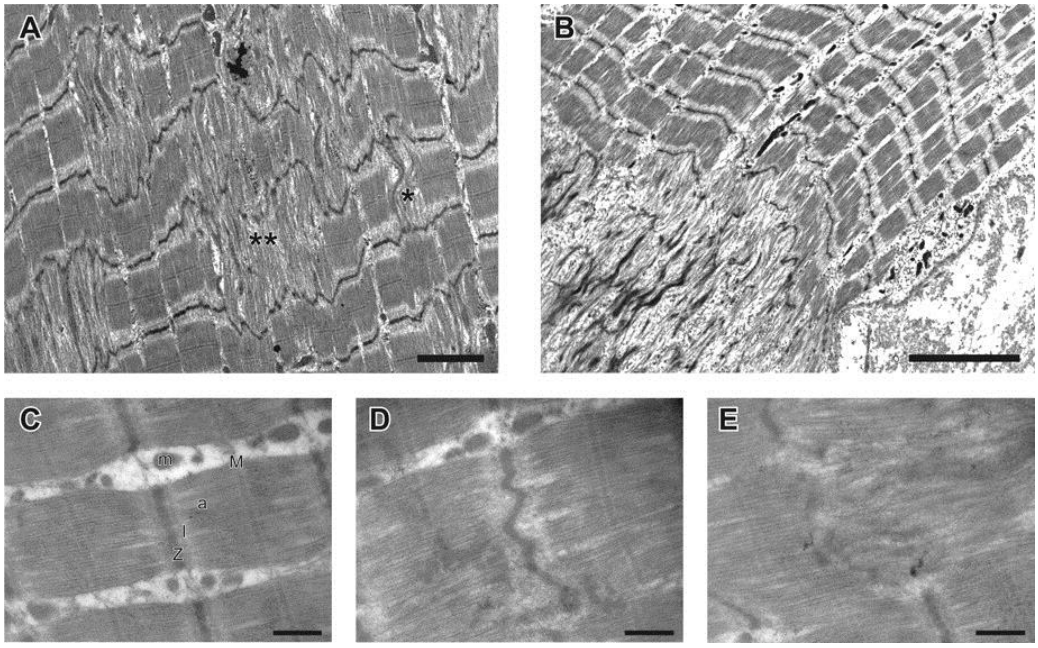

Figure 1. Sample electron micrographs of exercised skeletal muscle illustrating six categories of muscular disruption (Lauritzen, Paulsen, Raastad, Bergersen, & Owe, 2009).

Panel A illustrates focal (*) and moderate (**) disruption and Panel B illustrates extreme disruption. Panel C exemplifies a Z-line (Z), A band (a), I band (I), M line (M) and mitochondria (m) in a sarcomere with intact Z-line. Panel D and E show disrupted and destroyed Z lines respectively.

While transient changes in calcium (Ca^{2+}) are essential in excitation-contraction (E-C) coupling, a structural disturbance of the sarcolemma allows an influx of Ca^{2+} in to the fibre at the site of injury (Gissel, 2006); resulting in a loss of Ca^{2+} homeostasis (Armstrong, Warren, & Warren, 1991). Elevated intracellular Ca^{2+} can activate intrinsic degradative pathways in the muscle fibre; including the phospholipase A_2 (PLA_2) cascade (which leads to the production of arachadonic acid and its subsequent conversion to prostaglandin H_2 (PGH_2), for example), and the Ca^{2+} dependant protease calpain (Armstrong et al., 1991). Calpain is associated with degradation of a variety of protein substrates including cytoskeletal (such as desmin and α-actinin), myofibrillar, and membrane proteins. Gissel (2006) describes the increase in these degradation processes as activating a 'vicious cycle' by promoting further increases in cellular membrane permeability, and thus causing additional influx of Ca^{2+}. Excessive influx of Ca^{2+} and subsequent calpain activation is

therefore thought to contribute to Z-line streaming and the E-C uncoupling that occurs immediately after exercise (Gissel, 2006). Indeed, ultrastructural deformities of the SR and/or t-tubules is accompanied by depressed rates of Ca^{2+} sequestering, and therefore results in less Ca^{2+} for each excitatory action potential (Newham et al., 1983) and a decrease in the degree of actin-myosin interaction (Vollestad & Sejersted, 1988). This disruption in E-C coupling affects the ability of muscle to activate intact force-generating structures and produce maximal force.

During exercise, contracting skeletal muscle increases the production of endogenous free radicals and this was first reported more than 30 years ago (Davies, Quintanilha, Brooks, & Packer, 1982). Despite initial indications that mitochondria are the predominant site for free radical production (including reactive oxygen and nitrogen species; RONS) during physical activity, a number of alternative sites have been identified; including the SR, t-tubules and the plasma membrane (Powers & Jackson, 2008). In addition, increased PLA_2 activity has been reported to stimulate RONS generation above normal rates (Powers & Jackson, 2008). Therefore, processes elevating intracellular Ca^{2+} and the calcium-dependent PLA_2 cascade previously described are likely to exacerbate free radical production. Typically, free radicals are well controlled by antioxidant molecules and enzymes; however, excessive production may overwhelm and exceed protective mechanisms and the body's antioxidant capacity. Owing to this change in redox balance, there is an ensuing increase in oxidative stress caused by augmented levels of lipid peroxidation, protein oxidation and deoxyribonucleic acid (DNA) damage (Powers & Jackson, 2008). Whilst these reactions assist in the destruction and removal of damaged and necrotic cells, there may inevitably be some degree of oxidation to surrounding non-damaged cells, thereby exacerbating muscle damage (Cheeseman & Slater, 1993). Additionally, a redox balance in favour of a pro-oxidative state is known to reduce muscle force production (Reid, 2001).

Mechanical and metabolic alterations during initial muscle damage manifest themselves in muscle fibre disruption, impaired Ca^{2+} homeostasis and transport, faulty E-C coupling, abnormal cellular energetics, and the inability of the muscle to produce tension (Stauber, 1989). While these various autogenetic processes continue, this initial mechanical and metabolic damage causes a cascade of events leading to secondary damage.

2.2.1.2 Secondary response

Initiated by the disruption of intracellular Ca^{2+} homeostasis, secondary muscle damage is characterised by an inflammatory response, which begins within one hour of exercise (Smith, Kruger, Smith, & Myburgh, 2008; Tidball, 2005). Neutrophils are the first cells to accumulate at the injury site; their presence peaking approximately 24-48 h post exercise but can remain elevated for several days (Fielding et al., 1993). There are a number of chemotactic factors thought to promote neutrophil invasion into the muscle, including the presence of calpain (Belcastro, Shewchuk, & Raj, 1998), peptides fragments from the damaged tissue itself, and inflammatory cytokines such as tumour necrosis factor α (TNF-a), interleukin (IL) 1, and IL-6 (Close, Ashton, McArdle, & Maclaren, 2005). Indeed, evidence suggests that IL-6 is the first cytokine that appears in the circulation during and after exercise (Petersen & Pedersen, 2005) and is a major mediator in the acute phase response (Heinrich, Castell, & Andus, 1990; Kendall & Eston, 2002). The primary role of neutrophils is thought to be in the clearance of necrotic debris through phagocytosis (Smith et al., 2008), however, given that it is unable to distinguish between foreign and host antigens, it may also destroy healthy cells in the process (Pyne, 1994).

Neutrophils are also implicated in the recruitment of circulating macrophages to the injury site (Li, Cummins, & Huard, 2001). The number of macrophages increase approximately 24 h post exercise and are apparent for up to 14 days (Tidball, 2005, 2011). There are three subtypes, two of which occur in skeletal muscle tissue; most monocytes and macrophages are labelled M1 (or $ED1^{+}$) and appear in injured muscle first, and M2 (or $ED2^{+}$) macrophages are 'resident' in tissue (Clarkson & Sayers, 1999; Tidball, 2011). At present, there is a poor understanding regarding the functions of macrophages following muscle injury. However, in addition to neutrophils, phagocytic macrophages are thought to play a major role in the removal of cellular debris (Smith et al., 2008). Moreover, macrophages are also associated with muscle repair and regeneration (Tidball, 2005). Indeed, non-phagocytic M2 macrophages may serve as a major source of growth factors (such as insulin-like growth factor 1) that facilitate regeneration processes (Smith et al., 2008).

Whilst they are involved in the elimination of damaged tissue, neutrophils and macrophages also secrete pro-inflammatory cytokines (Clarkson & Hubal, 2002; Smith et al., 2008) and produce large quantities of free radicals through phagocytosis (Clarkson & Hubal, 2002; Close et al., 2005). Consequently, the proliferation of inflammatory cells in the hours and days following eccentric exercise may exacerbate existing damage elicited from the primary mechanical insult. This could explain the observed increase in ultrastructural damage in the days post muscle injury (Clarkson & Sayers, 1999).

The secondary inflammatory response is extremely complex, given that many inflammatory cell types and cytokines are involved in several (sometimes pleiotropic) functions and many processes may occur simultaneously. Certainly, besides their roles in phagocytosis, the destruction of muscle fibres and production of free radicals, immune cells also play a pivotal role in mediating muscle repair (Smith et al., 2008).

2.2.1.3 Summary of proposed mechanisms of exercise-induce muscle damage

While well-researched, the sequence of events associated with EIMD are not well defined, nor fully understood. Traditionally, it is thought that mechanical and metabolic damage are initially responsible for primary EIMD, and cause the subsequent secondary 'phagocytic' phase important in the removal of damaged cells and in stimulating regeneration of injured tissue (Armstrong, 1990). This model of muscle damage is summarised in Figure 2. However, it is likely that the stages of muscle damage described here overlap enormously (Kendall & Eston, 2002) and that no single mechanism is responsible for the observed effects following eccentric exercise (Clarkson & Sayers, 1999). Moreover, our understanding is further challenged by the different methods used to investigate EIMD, and these are discussed in the following section 2.2.2.

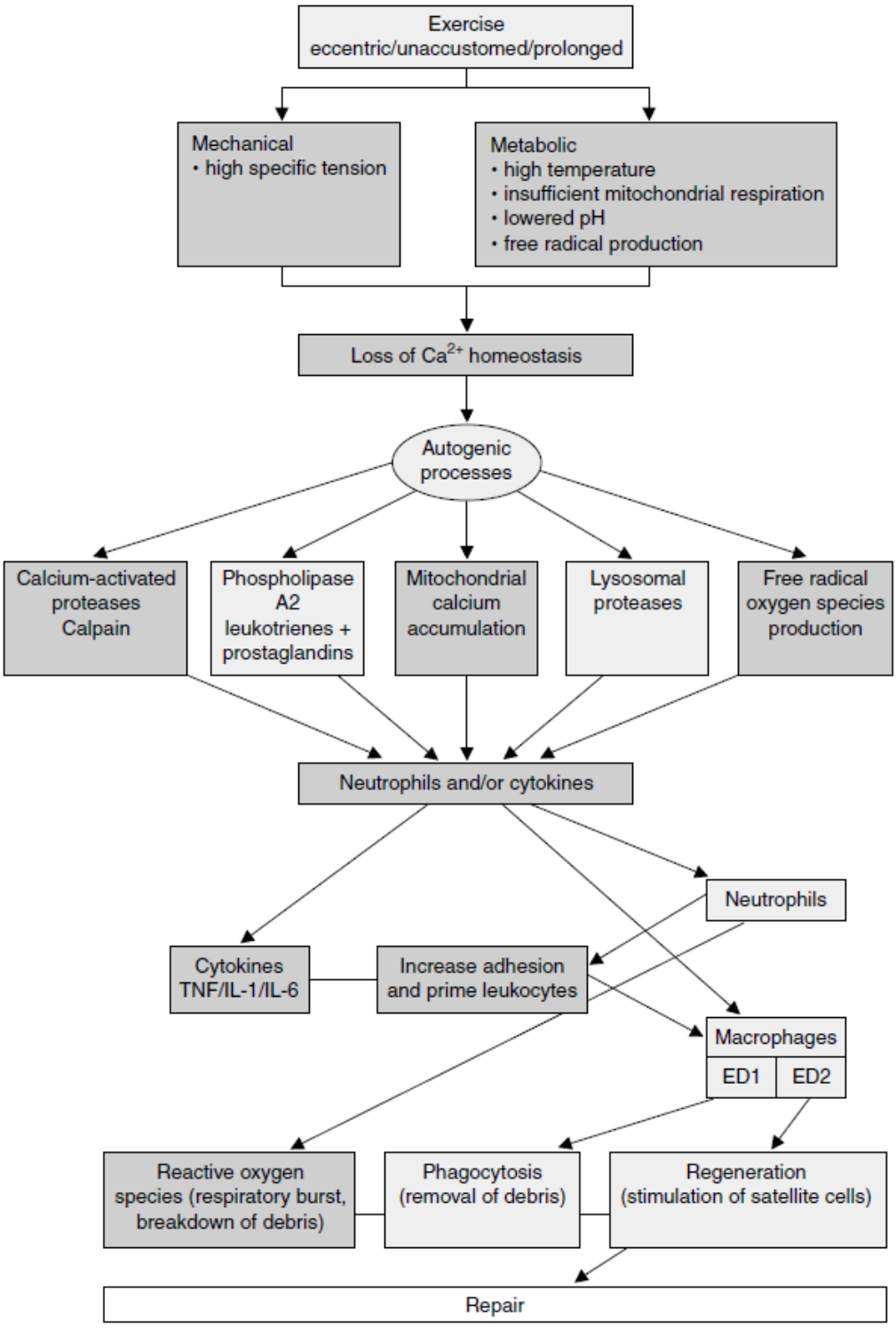

Figure 2. Illustration of a simple model of the muscle damage and repair cycle (Kendall & Eston, 2002).

Ca^{2+}, calcium; TNF, tumour necrosis factor; IL-1, interleukin 1; IL-6, interleukin 6. Dark grey boxes illustrate areas where oestrogen may play a potential inhibitory role (discussed in section 2.2.3.3).

2.2.2 Markers of exercise-induced muscle damage

Various markers are used in EIMD research and findings appear to depend heavily upon the measure investigated (Bloomer, 2007). Few direct indices can determine muscle damage and tend to involve muscle biopsy and lengthy histological staining techniques to determine myofibrillar disruption. With the intrusive nature of biopsies, the fact that they represent only a small fraction of the involved muscle, and the potential for this procedure to further exacerbate damage (Warren, Lowe, & Armstrong, 1999), many studies rely on surrogate markers of muscle damage. These include measures of muscle function, measures of soreness and stiffness, and the appearance of blood indices associated with EIMD. A major challenge in EIMD assessment is the non-uniform time frame of appearance and disappearance (as illustrated in Figure 3), and the lack of agreement or correlation between these indices. Therefore, it is important to understand the temporal characteristics of markers of EIMD.

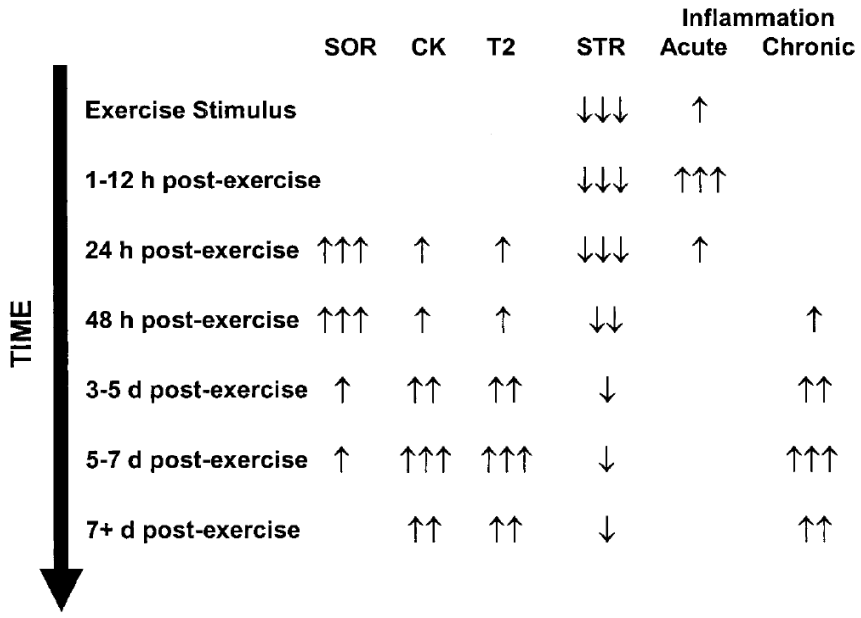

Figure 3. Time course of changes after maximal eccentric exercise (Clarkson & Hubal, 2002).

One arrow, minor increase/decrease; two arrows, moderate increase/decrease; three arrows, large increase/decrease; SOR, soreness; CK, creatine kinase; T2, magnetic resonance imaging T2 signal intensity; STR, strength.

2.2.2.1 Muscle function

Muscle functionality is considered to be the most important measure of EIMD for athletic populations, given that the ability to generate force and maintain optimal performance is of primary concern. Maximal isometric voluntary contraction (MVC) peak force or torque is suggested to be the best measure of muscle damage resulting from eccentric contraction, and provides the primary means for determining muscle function (Warren et al., 1999). Strength has been shown to decline by 10-65%, depending largely on the nature and intensity of eccentric exercise performed (Clarkson & Hubal, 2002), beginning immediately post exercise and persisting for several days or even weeks (Howell, Chleboun, & Conatser, 1993). Warren, Ingalls, Lowe, and Armstrong (2002) estimated that most (~75%) of force loss is attributed to E-C coupling failure and the remainder is due to damage of force-generating and/or force–transmitting structures within the muscle; at least in the subsequent 2-3 days following eccentric exercise.

While MVC assessment is considered the most valid and reliable indirect marker of EIMD, normal human muscle movement rarely involves isolated muscle actions. Typically, dynamic muscle function involves a sequence of eccentric stretch, isometric coupling, and concentric contraction; referred to as the stretch shortening cycle (Komi, 2000). Vertical jumping activities can assess this type of muscle function as they might occur in the sporting context; including the countermovement jump (CMJ), drop jump (DJ) and squat jump (SJ). The DJ also provides the opportunity to assess reactive strength, i.e. the ability to reverse a movement from eccentric to concentric contraction at high speed (Young, Wilson, & Byrne, 1999). Jumping performance appears to deteriorate for approximately 72 h post EIMD, though the SJ appears most affected by EIMD (Byrne & Eston, 2002a; Chatzinikolaou et al., 2010).

Other performance measures pertinent to sport and exercise activity have been used to assess muscle function following EIMD. Sprint performance is particularly important for intermittent and repeated-sprint sports such as soccer, rugby and field hockey. While no detrimental effect on sprint time has been observed previously (Semark, Noakes, St Clair Gibson, & Lambert, 1999), sprint performance has been demonstrated to be reduced following EIMD by others (Cockburn, Bell, &

Stevenson, 2013; Highton, Twist, & Eston, 2009; Keane, Salicki, Goodall, Thomas, & Howatson, 2015; Twist & Eston, 2005). In addition, given that range of motion is reduced following EIMD (Warren et al., 1999), and the observed relationship between hamstring stiffness and susceptibility to muscle damage (Chen et al., 2011; McHugh et al., 1999), a number of studies have measured flexibility to assess EIMD (for example Ormsbee et al. (2015) and Vanderthommen, Chamayou, Demoulin, Crielaard, and Croisier (2015)). Decreases in flexibility are observed immediately following muscle damage and are evident for several days (Chen et al., 2011; Eston, Rowlands, Coulton, McKinney, & Gleeson, 2007). Flexibility is considered to be key to successful performance in a number of sports, including dance (Liederbach, 2000), and identifying changes in this marker during recovery from EIMD is important to these populations.

Measurement of MVC continues to be the primary means of determining muscle function following EIMD. However, it is apparent that EIMD has a multi-faceted effect on muscle functionality, and assessment of other dynamic, and exercise specific measures, is of interest. Indeed, these would bear more weight on the consequences of damage to athletic populations.

2.2.2.2 Muscle soreness and swelling

There is an algestic response following EIMD in the form of delayed onset muscle soreness (DOMS), typically appearing and peaking 24-48 h post EIMD, remaining elevated for several days, and eventually disappearing approximately 5-7 days post EIMD (Armstrong, 1984; Cleak & Eston, 1992a; Ebbeling & Clarkson, 1989; Proske & Morgan, 2001; Tee et al., 2007). While DOMS is perhaps considered to be the most familiar marker of EIMD, its aetiology is not wholly understood. Some authors have suggested that soreness could be more related to the inflammatory response than to the muscle damage itself (MacIntyre, Reid, Lyster, Szasz, & McKenzie, 1996; Smith, 1991). It is thought that the increase in tissue osmotic pressure associated with inflammation results in the sensitising of afferent nociceptive fibres, subsequently magnifying feelings of soreness and pain (Kraemer, French, & Spiering, 2004; Proske & Morgan, 2001). A significant relationship between DOMS and inflammation (evidenced by IL-6) has been reported following

acute eccentric EIMD in the quadriceps (MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2001). However, in contrast, no correlation between muscle soreness and inflammation has been demonstrated previously (Malm et al., 2000) and indeed DOMS has been shown to correlate poorly with other indicators of EIMD (Warren et al., 1999). Nevertheless, given that DOMS may influence adherence to exercise training and subsequent performance potential, it is an important consequence and marker of EIMD.

Administering of a visual analogue scale (VAS) is a common and simple method used to assess DOMS subjectively. The VAS has demonstrated excellent reliability (Bijur, Silver, & Gallagher, 2001) and is sensitive to changes following muscle-damaging exercise (Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Cockburn et al., 2013; Howatson et al., 2010). Algometry has also been shown to be a reliable measure (Nussbaum & Downes, 1998) and has been used as an objective means of monitoring symptoms of experimental DOMS and pain following EIMD in a number of studies (Clifford, Bell, West, Howatson, & Stevenson, 2016; Connolly, McHugh, & Padilla-Zakour, 2006; Levers et al., 2015; Peschek, Pritchett, Bergman, & Pritchett, 2014).

In addition to DOMS, muscle swelling and an increase in muscle circumference are also thought to indicate acute inflammation (Smith, 1991). This is as a result of an accumulation of fluid from the bloodstream into the interstitial spaces with inflammation; exceeding lymphatic drainage capacity and causing oedema (Chleboun, Howell, Conatser, & Giesey, 1998; Connolly, Sayers, & McHugh, 2003; Nosaka & Clarkson, 1996). While no association between soreness and swelling has been observed post EIMD (Cleak & Eston, 1992b; Yu, Liu, Carlsson, Thornell, & Stal, 2013) others suggest swelling might contribute to the sensation of pain and soreness (Friden, Sfakianos, Hargens, & Akeson, 1988; Lieber & Friden, 1999). In fact, it has been suggested that tissue swelling may be more important to the production of pain and inflammation than mechanical damage to the muscle fibre (Stauber, Clarkson, Fritz, & Evans, 1990). However, muscle swelling typically appears after 48 h and peaks up to 10 days post EIMD (Clarkson, Nosaka, & Braun, 1992). Given that the peak in muscle soreness usually occurs before the onset of swelling, the relationship between these parameters remains unclear (Clarkson & Hubal, 2002). Due to limited and costly measurement techniques to directly

determine intracellular fibre swelling, many studies measure muscle swelling using either sonography or a change in limb circumference (Howell et al., 1993; Nosaka & Clarkson, 1996; Yu et al., 2013).

2.2.2.3 Biochemical markers

Increases in plasma activity of intramuscular proteins (for instance myoglobin, lactate dehydrogenase; LDH, and creatine kinase; CK) is associated with myofibrillar damage and increased permeability of the sarcolemma (Howatson & van Someren, 2008). The intramuscular protein most commonly used as an index of EIMD is CK, perhaps given its large response following exercise (Clarkson & Hubal, 2002). Since it is a relatively large molecule, it does not easily permeate damaged membranes, and therefore has a delayed response compared to other intramuscular proteins. Typically, CK concentrations increase within hours, peak ~24-48 h, and remain elevated for ~72 h after muscle damage (Brancaccio, Maffulli, & Limongelli, 2007; Mougios, 2007). However, the response varies widely, and there is evidence to suggest that the peak and elevation of CK can extend beyond this period following eccentric exercise (Serrao et al., 2003). Relative to the exercise performed, EIMD can result in modest elevations above normative levels of CK, but can also reach several thousand $IU \cdot L^{-1}$ (Cooke, Rybalka, Stathis, Cribb, & Hayes, 2010; Howatson et al., 2010; Leeder et al., 2014). As well as the intensity and volume of exercise influencing CK, there is also large individual variability with this marker (Howatson, Hoad, et al., 2012). This has been attributed to factors including training status and supposed high and low responders (Brancaccio et al., 2007); with genetic variations in the coding of myofibrillar proteins influencing the phenotypic response to muscle-damaging exercise (Baird, Graham, Baker, & Bickerstaff, 2012; Clarkson et al., 2005).

Measurement of systemic markers of inflammation is common in EIMD studies, such as the appearance of cytokines, leukocytes, and C-reactive protein (CRP). CRP is predominantly released from hepatocytes, and IL-1, IL-6, and TNF-α have been identified as regulators of its production (Yoshida et al., 2002). Consequently, CRP is recognised to increase within a few hours and peak approximately 24 h post exercise; after the appearance of inflammatory cytokines. Indeed, CRP was shown

to increase by ~1100% 24 h following repeated bouts of anaerobic exercise (Meyer, Gabriel, Ratz, Muller, & Kindermann, 2001). As with a number of mediators of the inflammatory processes, CRP has been shown to promote both anti- and pro-inflammatory activities. It has been implicated in the increased release of the anti-inflammatory cytokine IL-10, whilst also increasing the release of pro-inflammatory cytokines such as IL-1 and TNF-α (Black, Kushner, & Samols, 2004). While these anti- and pro-inflammatory properties are necessary for the process of muscle repair, caution should be taken regarding the interpretation of CRP expression as it can either dampen or enhance the inflammatory response depending on the circumstance (Black et al., 2004). However, the relatively long plasma half-life (18-20 h), stability, and limited circadian variation of CRP make it a sensitive and accurate marker of systemic inflammation (Pepys & Hirschfield, 2003). Additionally, more sensitive measurement techniques for detecting CRP have also been developed (high-sensitivity CRP; hsCRP) which make measurement and comparison of very low CRP levels in blood possible (Hsieh et al., 2013).

A great deal of biochemical measures to determine the oxidative stress response following exercise as well as antioxidant capacity are available. These include assessment of oxidants (such as hydroxyl radicals and superoxide anions), antioxidants (such as total antioxidant capacity (TAC) and oxygen radical absorbance capacity (ORAC), oxidation products (such as protein carbonyls and lipid hydroperoxides (LOOH)) and antioxidant/pro-oxidant balance (such as oxidised/reduced glutathione ratio; GSSG/GSH). It is important to note that some methods used to quantify antioxidant capacity and oxidative stress require lengthy analysis, lack validity due to various limitations in assay techniques (such as interaction with other compounds and variability among instruments), and/or are costly (please refer to Powers, Smuder, Kavazis, and Hudson (2010)). These markers and the methods used to assess them are not discussed here, and the reader is directed to previously published reviews by Powers et al. (2010), Powers and Jackson (2008) and Jackson (1999).

2.2.2.4 Summary of markers of muscle damage

There is a plethora of measures that are used to assess EIMD and recovery, each with various strengths and limitations. In addition, rates of appearance and recovery vary between indices of EIMD (as illustrated in Figure 3). Some studies choose to measure a small number of markers of muscle damage; at times blood indices in the absence of performance and/or muscle soreness measures (for instance Bell, Walshe, Davison, Stevenson, and Howatson (2014) and Coombes and McNaughton (2000) or vice versa (for instance Connolly et al. (2006) and Kuehl, Perrier, Elliot, and Chesnutt (2010)). Studies which have measured several indices of damage and that explore a number of symptoms associated with EIMD throughout recovery are more likely to identify the damage response and substantiate any effects of an intervention. Therefore, muscle damage research should employ a multi-dimensional test panel, consisting of both biochemical and performance related parameters (Kraemer & Ratamess, 2005), particularly when lacking direct measurement. Nevertheless, force/ torque producing capability remains the best method of quantifying muscle injury (Warren et al., 1999).

2.2.3 Determinants of muscle damage

It is important to acknowledge a number of factors that influence the EIMD response, which must be considered when interpreting the literature. These have the potential to affect the magnitude and duration of muscle damage and therefore the potential efficacy of interventions to reduce EIMD.

2.2.3.1 Exercise type

The magnitude of EIMD varies relative to the mode, volume, intensity, and duration of exercise (Hyldahl & Hubal, 2014; Proske & Morgan, 2001); each of which indirectly represent the muscle mass involved in the activity. Since contracting muscle *per se* is thought to be responsible for instigating muscle damage, it is perhaps unsurprising that increasing the volume, intensity and duration of exercise results in a concomitant increase in damage and the associated symptoms. For instance, eccentric downhill running typically results in 10-30% force loss (Eston,

Finney, Baker, & Baltzopoulos, 1996), whereas maximal eccentric actions of the elbow flexors generate 50-65% force loss (Newham, Jones, & Clarkson, 1987) post exercise. Moreover, prolonged concentric (cycling) exercise has been shown to result in increased concentrations of CK (Saunders, Kane, & Todd, 2004; Saunders, Moore, Kies, Luden, & Pratt, 2009). However, as described previously (section 2.2.1), whilst all forms of strenuous exercise have the potential to illicit pain (Proske & Morgan, 2001), novel and eccentric-biased actions result in greater levels of EIMD (Gibala et al., 2000; Penailillo et al., 2013).

The majority of research studies investigating EIMD use isolated muscle groups to induce eccentric muscle damage, primarily with isokinetic contractions of the knee extensors and elbow flexors (Jamurtas et al., 2005). However, it has been reported that EIMD is greater using elbow flexors compared to knee extensors, perhaps attributable to an adaptation to eccentric contractions in the lower limbs through daily activities such as walking downstairs or downhill (Jamurtas et al., 2005). Therefore, interpreting results based on divergent eccentric protocols using different muscle groups is challenging. Moreover, whilst the ability of single muscle group protocols to effectively induce a large muscle damage response is advantageous, these activities are not entirely representative of the exercise encountered by many athletic populations. Therefore, the application of findings in response to such protocols is limited. Research which adopts whole-body eccentric-biased exercise protocols to elicit muscle damage has far greater ecological validity; for instance, downhill running, plyometrics, sport-specific paradigms, and repeated-sprint based exercise. Results from such studies are more pertinent to the exercisers themselves and to practitioners and exercise scientists working with athletic populations in the field. Certainly, more research using sport-specific exercise paradigms to induce muscle damage is warranted.

2.2.3.2 Training status

It is recognised that the training status of an individual will play a role in EIMD as differences in metabolic and muscular physiology will create different responses to damage (Tee et al., 2007). Skeletal muscle has the ability to rapidly adapt, and indices of muscle damage are attenuated following a second bout of potentially

damaging exercise. Although the mechanisms are not fully understood, this commonly termed repeated bout effect (RBE) has been attributed to neural, mechanical or cellular adaptations (including adaptation in excitation-contraction coupling and the inflammatory response); or, more likely, a combination of these (Howatson & van Someren, 2008; McHugh, 2003). Thus, while muscle damage and the associated oxidative stress and inflammation are often considered negative, they may be a crucial part of the adaptive process (Sousa, Teixeira, & Soares, 2014) and an individual is to some extent protected (even following a single bout of an exercise stimulus) against damage with a subsequent bout (Howatson, Van Someren, & Hortobagyi, 2007; McHugh, 2003). Moreover, as eluded to previously, novel eccentric-biased exercise results in a greater magnitude of damage. Evidently, trained populations, who are likely adapted due to repeated exposure to strenuous exercise and physiological stress, might display attenuated responses to such stimuli compared to untrained populations (Barnett, 2006; McHugh, 2003; Morton, Kayani, McArdle, & Drust, 2009).

Research suggests that the exercise stimulus must be appropriate in order to elicit skeletal muscle damage in the subject population in question. For instance, though there is evidence to suggest that well trained individuals experience appreciable damage from a strenuous bout of exercise (Leeder et al., 2014), the stimulus must be of adequate intensity and volume to effectively induce muscle damage (Wilson, Lowery, et al., 2013) or else an attenuation or improvement from an intervention is unlikely to be achieved or indeed detectable. As a result, many studies recruit untrained participants with the intention of ensuring robust changes in muscle damage following exercise. However, whether the findings can be applied to trained populations is questionable given the aforementioned habituation and adaptation associated with strenuous exercise (Pasiakos, Lieberman, & McLellan, 2014).

2.2.3.3 Sex differences

The literature examining the differences in susceptibility of EIMD between men and women is equivocal. Some investigations have suggested that there are either no sex differences or that females are in fact more prone to EIMD (Dannecker, Knoll, & Robinson, 2008; Rinard, Clarkson, Smith, & Grossman, 2000; Sayers & Clarkson,

2001). In contrast, others report that females appear to experience less muscle damage following eccentric exercise compared to males (Dannecker et al., 2012; Kendall & Eston, 2002; MacIntyre, Reid, Lyster, & McKenzie, 2000; Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015; Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008; Stupka et al., 2000; Wolf et al., 2012). Though the cause for the discrepancies across sexes is largely unknown, a number of proposals have been put forward as tentative explanations. For instance, the differences in muscle fibre cross-sectional area (Miller, Macdougall, Tarnopolsky, & Sale, 1993) are considered to influence EIMD; with a greater muscle mass involved during exercise and the potential to perform more work, males may be subjected to more structural damage. However, predominantly (and although the mechanisms remain to be fully elucidated), hormonal differences are suggested to explain an attenuated damage response in females. Certainly, rodent models have provided compelling evidence of the efficacy of oestrogen in reducing EIMD, inflammation, and assisting muscle repair (Enns, Iqbal, & Tiidus, 2008; Enns & Tiidus, 2008; Tiidus, Deller, & Liu, 2005). The processes of muscle damage, which are thought to be influenced by oestrogen are highlighted in Figure 2.

Specifically, oestrogen is thought to have antioxidant properties (Tiidus et al., 2005; Wolf et al., 2012) as oestrogens, similar to vitamin E, display a hydroxyl group on their phenolic ring (Tiidus et al., 2001). It is thought that oestrogen donates the hydrogen atom to lipid peroxyl radicals, limiting lipid peroxidation in the cell membrane (Kendall & Eston, 2002). Given that it is lipophilic, oestrogen is also suggested to have membrane stabilising properties, by direct incorporation into cell membranes in a similar way to cholesterol; thus optimising membrane fluidity and polyunsaturated fatty acid arrangement (Tiidus, 2003). This may explain why females have been shown to demonstrate lower basal circulating concentrations of CK, and a lower CK response following exercise compared to males (Wolf et al., 2012). Indeed, Carter, Dobridge, and Hackney (2001) have previously reported a moderate negative correlation ($r = -0.43$) between total oestrogen levels and CK following a bout of downhill running. Moreover, oestrogen may exhibit an inhibitory effect on the inflammatory cascade in skeletal muscle (evidenced by an attenuated leucocyte infiltration (Tiidus, 2003; Tiidus et al., 2005)), compared to males who appear to demonstrate a more prolonged damage response (Fragala et al.,

2011; Heavens et al., 2014). Indeed, oestrogen has been shown to inhibit nuclear factor kappa beta intracellular localisation (central mediator of the acute inflammatory response), consequently limiting pro-inflammatory gene expression (Ghisletti, Meda, Maggi, & Vegeto, 2005). Additionally, antioxidant and membrane stabilising characteristics may limit the fluctuations of intracellular Ca^{2+} homeostasis and thus diminish calpain activation or influence other membrane related neutrophil capture or adhesion mechanisms (Tiidus et al., 2005; Wolf et al., 2012).

These data demonstrate that oestrogen has a potentially multifactorial influence on skeletal muscle damage. However, sex differences are complicated by factors beyond oestrogen. Indeed, hormone levels vary among women and within women across their individual menstrual cycles. Recent evidence suggests that EIMD and recovery may differ between menstrual cycle phases (Markofski & Braun, 2014; Sipaviciene, Daniuseviciute, Kliziene, Kamandulis, & Skurvydas, 2013). Despite this, many investigations in females conduct testing during the early follicular phase where oestrogen is at its lowest levels, though still higher than males (Stachenfeld & Taylor, 2014). While this may conveniently control for hormonal effects, this period represents only a quarter of the menstrual cycle (Stachenfeld & Taylor, 2014), and the application of the results reported in such studies is therefore limited to females in this phase of the menstrual cycle. If oestrogen does indeed play a role in the attenuation of muscle damage and accelerated recovery in females, testing in the luteal phase (where oestrogen is markedly elevated) may better observe these effects. However, many studies examining EIMD using female subjects do not report how changes in the menstrual cycle were (if at all) accounted for.

Moreover, contraceptive use appears to implicate the severity of EIMD in females. The most biologically active and abundant endogenous form of oestrogen is 17β-oestradiol (Savage & Clarkson, 2002; Stachenfeld & Taylor, 2014) and concentrations exhibit large individual variations (Guerrero et al., 1976). Some forms of contraception expose the body to synthetic forms of oestrogen, and naturally occurring oestrogen may be found in lower quantities (Minahan et al., 2015). Consequently, non-contraceptive users (demonstrated to have on average twice the oestradiol levels in the early to mid-follicular phase) have been reported to receive more protection against EIMD and a more rapid recovery of strength

compared to oral contraceptive users (Minahan et al., 2015; Savage & Clarkson, 2002). While the inclusion of females using different forms of contraception is important to reflect the heterogeneity of this population, the lack of detail regarding participant contraceptive use is a limitation in the EIMD literature. Finally, it is important to note that some female athletes may become hypo oestrogenic with high levels of physical activity (Warren & Perlroth, 2001) and oestradiol has been reported to be reduced in physically active women compared to less active women (Mitsuzono & Ube, 2006). Moreover, energy deficiency may also suppress reproductive function with a concomitant reduction in oestrogen (De Souza & Williams, 2005). This should be considered when research is concerned with trained female populations, particularly those at risk of energy deficiency. Indeed, the effects of oestrogen may be less pronounced, and might explain why some have failed to identify sex differences in EIMD in athletic populations.

While speculation remains as to whether sex differences exist, a growing body of evidence suggests that oestrogen may help to maintain muscle membrane integrity consequent to muscle damage, and as a result, the initial physiological stress and ensuing recovery associated with EIMD in females is likely to differ compared to male populations. This highlights the difficulty in extrapolating the findings provided by male participants to female athletes. Indeed, the majority of research investigating EIMD to date has recruited male participants, and many investigations using both males and females combine sexes in treatment groups and fail to acknowledge the potential influence of sex. It is clear that while much more research is required regarding the potential influence of sex in muscle damage and recovery, the accurate reporting of menstrual cycle phase and contraceptive use of female participants is essential in order to develop our understanding. Certainly, while the effect of sex remains unclear, more research in females is necessary and researchers should take the same care in considering the hormone milieu as they do with any variable that is likely to influence their findings.

2.2.4 Evidence for exercise-induced muscle damage following dance

Research demonstrates that dance is a form of moderate-high intensity, intermittent and high skill activity with complex movement sequences (Beck, Redding, et al.,

2015; Wyon & Koutedakis, 2013). Most dance activities incorporate a number of eccentric muscle actions including jumps and high-impact landing tasks, a variety of postures and positions, as well as explosive forces (Paschalis et al., 2012; Westblad, Tsaifellander, & Johansson, 1995). Dance-type exercise might also include elements of sprint activity (Cohen, Segal, Witriol, & McArdle, 1982); that has been shown to induce muscle damage (Howatson & Milak, 2009; Keane, Salicki, et al., 2015). Moreover, dancers engage is many hours of daily training, which may be accompanied by additional fitness training, rehearsals and performances (Bronner, Codman, Hash-Campbell, & Ojofeitimi, 2016; Grove, Main, & Sharp, 2013; Twitchett et al., 2010; Weiss, Shah, & Burchette, 2008; Wyon, 2010). These daily demands may be expected for many consecutive weeks during a performance period (Grove et al., 2013). The workloads of pre-professional dancers as part of dance school programmes are also reported to be high, particularly prior to studio showings and exams (Bronner et al., 2016; Grove et al., 2013). These data demonstrate that the intensity and volume of exercise of dance populations can often be comparable to that of many elite athletes. However, while the training of many athletes is periodised, dancers do not have defined nor predictable seasons, and training does not typically allow for rest (Liederbach, 2000). Indeed, there is rarely an opportunity to have a full day to recover post dance performance (Allen & Wyon, 2008).

A number of authors recognise that individuals taking part in dance activity are at risk of muscle damage (Paschalis et al., 2012; Twitchett et al., 2010), and given the evidence regarding the demands of dance, this is certainly conceivable. Indeed, to reach the highest technical levels, there may be substantial repetition of the aforementioned eccentric activities during dance training. In addition, it has been suggested that just 1-2 mins of maximal dance exercise (such as a jump section in dance class) can lead to declines in muscle force production (Wyon & Koutedakis, 2013). Interestingly, reports suggest the highest percentage of injuries in female contemporary dancers are of a muscular nature (Angioi, Metsios, Koutedakis, Twitchett, & Wyon, 2009) and lower muscular power (a symptom of EIMD) has been associated with an increase in incidence and severity of injuries in female contemporary dancers (Angioi, Metsios, Koutedakis, Twitchett, et al., 2009; Koutedakis, Khaloula, Pacy, Murphy, & Dunbar, 1997). Despite this, at the time of

writing, only one study has investigated the muscle damage response following dance activity (Rodrigues-Krause et al., 2014). The authors sought to describe and compare highly trained ballet dancers' cardiorespiratory, muscle damage and oxidative stress responses during a ballet class and rehearsal. This study demonstrated that CK was elevated for 48 h following both class and rehearsal. Additionally, lipid peroxidation was elevated immediately post class but unaffected following rehearsal, whilst a decrease in the oxidised/reduced glutathione ratio (GSSG/GSH) observed at 48 h in relation to levels immediately post class and rehearsal suggested an improved redox state. Finally, the authors reported differences in CK and lipid peroxidation responses between class and rehearsal, however given that pre-exercise differences in these measures were not normalised, a true difference is questionable. This research indicates, for the first time, that dance activity appears to induce muscle damage and oxidative stress. However, there are a number of limitations associated with this study, namely in its design; with a lack of measurement of muscle function, and a failure to monitor markers of muscle damage and oxidative stress beyond 48 h. In addition, Rodrigues-Krause et al. (2014) identified the importance of investigating responses in dancers with different technical characteristics. Evidently, there are differences in the specific characteristics and demands relative to different dance genres. Evidence suggests that, owing to the many different contemporary dance techniques (Cunningham, Release, Graham and Limón for instance), contemporary dance demands a greater variety of physical and technical skill compared to ballet (Weiss et al., 2008). Certainly, while it is acknowledged that optimal recovery is important for dancers, the muscle damage elicited following different activities in dance populations, and the profile of recovery has not been adequately investigated nor defined.

2.2.5 Summary

Though the mechanisms are not wholly understood, EIMD is typically summarised as a primary response involving mechanical damage of the contractile protein elements at the myofibrillar level precipitated during the exercise bout, and a secondary inflammatory response that can exacerbate or prolong the initial damage (for detailed reviews the reader is directed elsewhere (Clarkson & Hubal, 2002;

Howatson & van Someren, 2008)). Resulting symptoms include increases in muscle soreness, limb girth and biochemical markers of damage, as well as decreases in muscular functionality; which can be relatively long-lasting. It has been shown that unaccustomed exercise, particularly involving eccentric contractions, results in the greatest damage (Gibala et al., 2000; Penailillo et al., 2013), and the mode, intensity, and duration of the exercise bout influences the extent of EIMD (Hyldahl & Hubal, 2014; Proske & Morgan, 2001). Moreover, whilst the evidence regarding sex differences in EIMD remains controversial, oestrogen has been implicated to some extent in an attenuated muscle damage response reported in females (Kendall & Eston, 2002). This makes the expectation tenable that the muscle damage response differs between the sexes and more research using female participants (where information is scarce) is essential in order to develop our understanding. The characteristics and demands of dance training suggest that dancers might be at risk of experiencing EIMD. However, surprisingly, only one study (Rodrigues-Krause et al., 2014) to date has investigated muscle damage following dance, and this study lacked assessment of muscle function. Therefore, the muscle damage response in dancers requires further detailed investigation and would have potentially wide-reaching applications to this population.

2.3 Nutritional interventions for recovery

Previously, this literature review has discussed the negative symptoms associated with EIMD. A prolonged muscle damage response is of particular concern given the potential to inhibit engagement in exercise training and performance required of athletic populations (Howatson, Hoad, et al., 2012).

Moreover, compounding damage over consecutive exercise sessions could contribute to muscle injury (Heavens et al., 2014). As a result, methods to reduce muscle damage and accelerate recovery from EIMD are widely sought. Perhaps the most common strategy investigated to attempt to reduce the symptoms of muscle damage is the influence of nutritional interventions (Howatson & van Someren, 2008). This section will provide an evaluation of the literature examining the use of two contemporary nutritional supplementation strategies on the attenuation of

muscle damage and their ability to enhance recovery in healthy humans; tart Montmorency cherry and whey protein hydrolysate.

2.3.1 Tart Montmorency cherry

2.3.1.1 Background

Both sweet and tart cherry varieties contain high levels of antioxidants such as melatonin, carotenoids, and several flavonoid groups including anthocyanins, as well as the flavonol quercetin (McCune, Kubota, Stendell-Hollis, & Thomson, 2011). Tart Montmorency cherries (MC) account for 75% of tart cherry production in the United States, and while the tart Balaton cherry cultivar has been shown to contain six times the total anythocyanin content, MC has higher levels of total phenolics (Kirakosyan, Seymour, Llanes, Kaufman, & Bolling, 2009). Moreover, though frozen tart cherry products have greater quantities of total anthocyanins (Kirakosyan et al., 2009; Ou, Bosak, Brickner, Iezzoni, & Seymour, 2012), tart cherry concentrate has higher levels of total phenolics, and a greater level of antioxidant and anti-inflammatory activity per serving when compared to frozen, canned or dried cherries (Keane, Bell, et al., 2015; Ou et al., 2012). Additionally, the polyphenolic compounds that MC contain result in higher ORAC values compared to several other antioxidant beverages such as Concord grape, acai, iced green tea, and blueberry juice (Bell, McHugh, Stevenson, & Howatson, 2013; Seeram et al., 2008) as illustrated in Figure 4. It is important to note that there is variation in the anthocyanin content of fruits owing to nutritional, environmental and seasonal differences (McCune et al., 2011), and the metabolism, absorption and subsequent bioavailability of anthocyanins is also influenced by gut microflora and the food structure (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Wallace, 2011). However, anthocyanin bioavailability in plasma and urine has been typically reported to peak 1-2.5 h post ingestion despite differences in the dose (for review please refer to Manach et al. (2005)). Specifically, phenolic compounds of MC have previously been shown to be most abundant in plasma in the 1-2 h post consumption (Keane, Bell, et al., 2015).

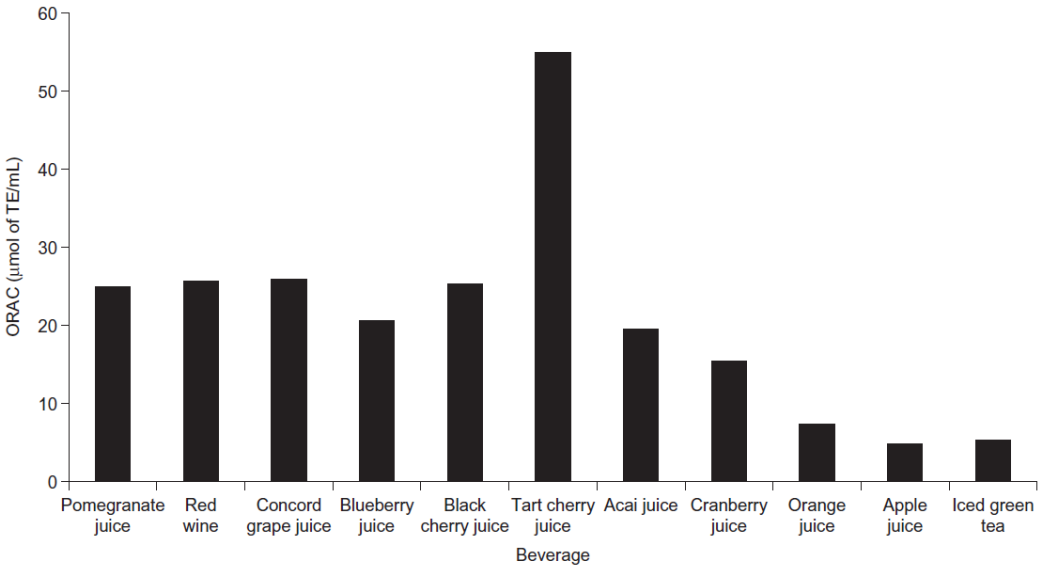

Figure 4. Comparison of antioxidant status of fruit juice beverages as assessed through oxygen radical absorbance capacity (ORAC; values sourced from Seeram et al. (2008) and Howatson et al. (2010)) (Bell et al., 2013).

As described in section 2.2.1.1, during exercise, initial muscle damage is thought to be caused by mechanical and metabolic disruption; owing in part to an increase in the production of free radicals (Davies et al., 1982; Powers & Jackson, 2008). At low concentrations, these can play an important role in gene expression, cell proliferation, apoptosis, and muscular contraction (Powers, Nelson, & Hudson, 2011). However, an excessive increase in free radical production can shift redox balance in favour of a pro-oxidative state which leaves lipids, protein and DNA susceptible to oxidation (Powers & Jackson, 2008). Whilst RONS and NO derivatives assist in the destruction and removal of damaged and necrotic cells, there may inevitably be some degree of oxidation to surrounding non-damaged cells, thereby exacerbating muscle damage (Cheeseman & Slater, 1993). Moreover, the secondary inflammatory response involving the degradation of damaged muscle by immune cells releases further pro inflammatory cytokines and free radicals (Clarkson & Hubal, 2002). The degree of oxidative stress appears dependent on the mode of exercise, and eccentric contractions may produce significantly greater amounts of RONS compared to concentric and isometric contractions; likely as a consequence of greater damage and inflammation associated with these activities

(Nikolaidis et al., 2012). Typically, endogenous antioxidant molecules and enzymes are able to protect against free radicals. However, their excessive production (for instance during exercise) may exceed antioxidant defence. This could have important implications for muscle damage and subsequent recovery. Consequently, MC has been proposed to be an effective recovery aid due to the high antioxidant content and anti-inflammatory properties present within it (Bell, Walshe, et al., 2014; Bell et al., 2015; Keane, Bell, et al., 2015; Kirakosyan et al., 2015; Seeram, Momin, Nair, & Bourquin, 2001; Wang, Nair, Strasburg, Chang, et al., 1999). Certainly, a number of studies (please refer to Table 2 and section 2.3.1.2) have demonstrated efficacy following damaging exercise (Bell, Stevenson, Davison, & Howatson, 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Connolly et al., 2006; Howatson et al., 2010; Kuehl et al., 2010; Levers et al., 2015).

Specifically, anthocyanins are able to scavenge free radicals directly as they are electron donors (Traustadottir et al., 2009). Therefore, given its anthocyanin content, it is perhaps unsurprising that tart cherry extracts have been implicated in the inhibition of lipid peroxidation *in vitro* (Mulabagal, Lang, DeWitt, Dalavoy, & Nair, 2009; Wang, Nair, Strasburg, Booren, & Gray, 1999a) and their efficacy has been shown to be comparable to commercial antioxidants (Wang, Nair, Strasburg, Booren, & Gray, 1999b; Wang, Nair, Strasburg, Chang, et al., 1999) and superior to that of vitamin E (Wang, Nair, Strasburg, Chang, et al., 1999). Moreover, anthocyanins are able to form cyanidin-DNA copigmentation complexes which are resistant to oxidative damage (Sarma & Sharma, 1999). Indeed, supplementation with an MC juice blend has been shown to reduce basal urinary excretion of oxidised nucleic acids following forearm ischemia-reperfusion in older men and women (Traustadottir et al., 2009). Intuitively, exogenous provision of these antioxidants are thought to improve redox balance and attenuate the oxidative stress caused by augmented levels of lipid peroxidation, protein oxidation and DNA damage.

Anthocyanins are also capable of indirectly attenuating the inflammatory response. Cyclooxygenase (COX) enzymes are responsible for converting arachidonic acid to prostaglandin H_2 (PGH_2), which is subsequently metabolised to produce bioactive

prostaglandins (such as prostaglandin E_2 (PGE_2)) as part of the inflammatory response (Tiernan, Imrhan, Prasad, Vijayagopal, & Juma, 2015). COX-1 is a constitutive enzyme and COX-2 is inducible in inflammatory conditions; therefore, COX-2 inhibition is desirable for the dampening of inflammation and pain (Tiernan et al., 2015). *In vitro*, MC cherry anthocyanins have shown both COX-1 and COX-2 inhibitory activities (Mulabagal et al., 2009; Seeram et al., 2001). In contrast, when measuring inhibitory effects of whole tart cherry products on COX enzyme activity, Ou et al. (2012) demonstrated that this was only evident with COX-1. Though the evidence *in vitro* is mixed, inhibition of COX enzymes may provide a possible mechanistic link to the observed benefits of MC supplementation for exercise recovery. Certainly, limiting the extent of acute inflammation during the neutrophil phase could attenuate muscle damage, pain and swelling (Smith et al., 2008).

In vitro studies have identified possible mechanisms of action for attenuation of EIMD with MC; namely in the dampening of oxidative tissue damage and the inflammatory response. It is widely acknowledged that these effects are attributed to anti-inflammatory properties of MC and the large quantities of antioxidants in the form of phenolic compounds and anthocyanins that they contain (Bell, Walshe, et al., 2014; Bell et al., 2015; Keane, Bell, et al., 2015; Kirakosyan et al., 2015; Seeram et al., 2001; Wang, Nair, Strasburg, Chang, et al., 1999). Importantly, the antioxidant and COX inhibitory activity of tart cherry extracts has been reported to be comparable to NSAIDs, (Mulabagal et al., 2009; Seeram et al., 2001; Wang, Nair, Strasburg, Chang, et al., 1999) and superior to that of vitamin E (Seeram et al., 2001; Wang, Nair, Strasburg, Chang, et al., 1999). As such, there is a growing interest in the use of such functional foods and natural antioxidant alternatives, in lieu of pharmacological drugs and analgesics, given the risk of suffering adverse effects (Ziltener et al., 2010). Moreover, it has been suggested that the antioxidant activities of fruits and vegetables are as a result of additive and synergistic effects of their phytonutrients, and that the same benefits are not evident with isolated dietary supplements (Liu, 2004). Despite this, there are few studies investigating the effects of MC on symptoms of EIMD *in vivo* (summarised in Table 1), and interestingly, while accelerated recovery of muscle function is often reported, the findings in regards to the potential mechanisms responsible for the observed effects are inconsistent.

Table 2. MC effects of recovery from EIMD.

				Supplement		Measure of EIMD		
Author	**Subjects**	**Design**	**Exercise**	**Dosage**	**Duration**	**Marker**	**Time points**	**Effect of MC**
Bell et al., 2016	16 semi-professional male soccer players (25 ± 4 y)	Randomised, double-blind, counter-balanced, placebo-controlled. Low polyphenolic diet throughout. (MC, *n*=8 vs isocaloric PL, *n*=8)	Adapted LIST	30 mL MC concentrate in 100 mL H_2O, twice per day (8am and 6pm)	8 days (4-day preload)	DOMS, IL-1-β, IL-6, IL-8, TNF-α, hsCRP, CK, LOOH, MVC, CMJ, 20 m sprint, and 5-0-5 agility	Pre, (and additional 1, 3, 5 h post exercise for blood indices), 24, 48, and 72 h post exercise	↓ DOMS and IL-6 ↑ MVC, CMJ, 20 m sprint, and 5-0-5 agility
Levers et al., 2015	23 healthy, resistance trained males (20.9 ± 2.6 y)	Randomised, double-blind, counter-balanced, placebo-controlled. (MC, *n*=11 vs rice flour PL, *n*=12)	10 x 10 70 % of a 1RM back squat exercise	One 480 mg powdered MC capsule daily (in the morning)	10 days (7-day preload)	DOMS (algometry), uric acid, creatinine, blood urea nitrogen, total protein, CK, complete blood count, AST, ALT, bilirubin, cortisol, testosterone, SOD, TAS, TBARS, nitrotyrosine, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, TNF-α, IFN-γ, GM-CSF, MVC	Baseline, pre exercise and 1, 24, 48 h post exercise	↓ testosterone and pairwise comparisons: ↓ DOMS, creatinine, total protein, bilirubin, cortisol, AST and ALT ↑ lymphocyte and white blood cell count
Bell et al., 2015	16 trained male cyclists (30 ± 8 y)	Randomised, double blind, counter-balanced, placebo-controlled. Low polyphenolic diet throughout. (MC, *n*=8 vs CHO matched and isocaloric PL, *n*=8)	Simulated road race lasting 109 mins (from 8am)	30 mL MC concentrate in 100 mL H_2O, twice per day (8am and 6pm) during pre-load. Day of exercise consumed 15 min post blood sample and 10 min prior to performance	8 days (4-day preload)	DOMS, IL-1β, IL-6, IL-8, TNF-α, hsCRP, LOOH, CK, MVC, cycling economy and 6-s peak cycling power	Baseline, (and additional pre exercise, and 0, 1, 3, 5 h post exercise for blood indices), 24, 48 and 72 h post exercise	↓ IL-6 and hsCRP ↑ MVC and cycling economy

Table 2. Continued

				Supplement		Measure of EIMD		
Author	**Subjects**	**Design**	**Exercise**	**Dosage**	**Duration**	**Marker**	**Time points**	**Effect of MC**
Bell et al., 2014	16 trained male cyclists (30 ± 8 y)	Randomised, double-blind, counter-balanced, placebo-controlled. Low polyphenolic diet throughout. (MC, *n*=8 vs CHO matched and isocaloric PL, *n*=8)	Simulated road race lasting 109 mins (from 8am) on 3 consecutive days	30 mL MC concentrate in 100 mL H_2O, twice per day (8am and 6pm)	7 days (4day preload)	LOOH, IL-6, TNF-α, IL-8, IL-1-β, hsCRP, CK	Baseline, pre exercise, and immediately post-trial on days 5-7	↓ LOOH, IL-6 and hsCRP
Bowtell et al., 2011	10 well trained male athletes (27.8 ± 1.6 y)	Randomised, double-blind, placebo-controlled, crossover (MC vs isocaloric PL)	10 x 10 single-leg knee extensions at 80% 1RM	30 mL MC concentrate twice per day (one in morning and one in afternoon after training)	10 days (7-day preload)	DOMS (PPT), CK, nitrotyrosine, hsCRP, TAC, protein carbonyls, MVC	Pre, 0, 24 and 48 h post exercise	↓ protein carbonyls ↑ MVC
Howatson et al., 2010	13 trained males and 7 trained females (MC group, 7M and 3F, 37 ± 13 y; PL group, 6M and 4F, 38 ± 5 y)	Pseudo-randomised, placebo-controlled (MC, *n*=10 vs PL, *n*=10)	Marathon	8 fl oz MC juice blend twice per day (morning and afternoon)	8 days (5-day preload)	DOMS, CK, LDH, IL-6, CRP, uric acid, TAS, TBARS and protein carbonyls, MVC	Baseline (for blood indices only), pre and 0, 24 and 48 h post-race	↓ IL-6, CRP, uric acid and TBARS ↑ MVC and TAS

Table 2. Continued

				Supplement		Measure of EIMD		
Author	**Subjects**	**Design**	**Exercise**	**Dosage**	**Duration**	**Marker**	**Time points**	**Effect of MC**
Kuehl et al., 2010	34 male and 17 female healthy runners (MC group, 19M and 7F, 38.2 ± 8.5 y; PL group, 15M and 10F, 32.2 ± 9.8 y)	Randomised, double-blind, placebo controlled (MC, *n*=26 vs PL, *n*=25)	Long distance running (26.3 ± 2.5 km in 24 h)	355 mL MC juice blend twice per day (both during race on race day)	8 days (7-day preload)	DOMS and satisfaction with supplement	Baseline, pre and post-race	↓ DOMS ↑ satisfaction
Connolly et al., 2006	14 males (22 ± 4 y)	Randomised, placebo-controlled, crossover (MC vs PL)	2 x 20 eccentric contractions of elbow flexors	12 fl oz MC juice blend twice per day (morning and evening)	8 days (3-day preload)	MVC, pain, muscle tenderness (algometry), relaxed elbow angle	Pre and 24, 48, 72 and 96 h post	↓ pain ↑ MVC

EIMD, exercise-induced muscle damage; M, male; F, female; PL, placebo; MC, Montmorency tart cherry; CHO, carbohydrate; 1RM, one-repetition maximum; LIST, Loughborough intermittent shuttle test; DOMS, delayed-onset muscle soreness; PPT, pain pressure threshold; CK, creatine kinase; LDH, lactate dehydrogenase; IL, interleukin; CRP, C-reactive protein; hsCRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; LOOH, lipid hydroperoxides; IFN-γ, interferon-γ; GM-CSF, granulocyte-macrophage colony-stimulating factor; AST, aspartate aminotransferase; ALT, alanine aminotransferase, SOD, superoxide dismutase; TAS, total antioxidant status; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; CMJ, countermovement jump; MVC, maximum voluntary contraction.

2.3.1.2 Evidence for the efficacy of tart Montmorency cherry for recovery

Connolly et al. (2006) were the first to investigate the effects of MC ingestion on EIMD and recovery. In a crossover study design, 14 males consumed either 12 fl oz (354.9 mL) of MC juice blend (consisting of tart cherry juice and apple juice in a proprietary ratio) or a placebo (PL) twice per day for 8 days surrounding an acute bout of 40 eccentric contractions of the elbow flexors. Compared to PL, pain and reductions in MVC were attenuated with MC consumption. In well-trained male athletes, Bowtell et al. (2011) also reported improved recovery of MVC following single-leg knee extensions at 80% 1RM with supplementation of 30 mL MC concentrate twice per day for 10 days compared to PL. However, in contrast to Connolly et al. (2006) and a number of studies (Bell et al., 2016; Kuehl et al., 2010; Levers et al., 2015), but in agreement with others (Bell et al., 2015; Howatson et al., 2010), muscle soreness was unaffected by MC ingestion (Bowtell et al., 2011). The authors suggested that this may have been as a result of the modest reduction in muscle soreness observed following exercise, which affected the ability to detect any effects of MC (Bowtell et al., 2011). Having said this, there was only a trend for reduced circulating CK (p = 0.055), and no other markers of muscle damage or inflammation were affected by MC. Interestingly, despite no change in total antioxidant capacity (TAC), serum concentrations of protein carbonyls were reduced with consumption of MC, suggesting that the accelerated recovery of muscle function was attributed to a reduction in oxidative damage rather than reduced muscle damage and inflammation (Bowtell et al., 2011).

It is important to note that the aforementioned studies (Bowtell et al., 2011; Connolly et al., 2006) adopted crossover study designs. It is possible that the participants experienced an attenuated EIMD response during the second exercise exposure due to the RBE (Howatson & van Someren, 2008; McHugh, 2003). This might have been exaggerated in the participants recruited by Connolly et al. (2006) given that they were able to continue exercise activity between bouts (though instructed not to use arms). While these studies attempted to minimise any RBE by allocating trials and limb order randomly (Connolly et al., 2006) or by systematic rotation (Bowtell et al., 2011), there is evidence to suggest that the RBE is carried to a contralateral limb (albeit the magnitude and duration of protection is less than that

of the ipsilateral limb) (Chen, Chen, Lin, Yu, & Nosaka, 2016; Howatson & van Someren, 2007). Therefore, the findings of these studies should be interpreted with this limitation in mind.

Though Bowtell et al. (2011) were unable to detect any effects of MC on hsCRP, others have demonstrated CRP/hsCRP and/or IL-6 are attenuated with MC compared to PL (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Howatson et al., 2010). The differences in these results are largely due to the different muscle-damaging exercise protocols employed. Bowtell et al. (2011) used single-leg eccentrically biased knee extensions which is arguably insufficient to elevate systemic inflammation; indeed, hsCRP did not increase following exercise. Similarly, a more recent study investigating the efficacy of supplementation with a daily 480 mg powdered MC capsule for 10 days reported no differences in an array of inflammatory markers compared to PL following a 100 barbell back squat protocol at 70 % 1RM (Levers et al., 2015). Compared to eccentric biased protocols, those that have observed reductions in CRP/hsCRP and/or IL-6 with MC utilised a marathon (Howatson et al., 2010), repeated bouts of high-intensity cycling on consecutive days (Bell, Walshe, et al., 2014; Bell et al., 2015), or repeated-sprint exercise (Bell et al., 2016) to induce muscle damage; all involving larger muscle mass and for a prolonged period of time. Certainly, it has been suggested that the positive effects of MC on recovery may be well suited to exercise with a high metabolic component (Bell, Walshe, et al., 2014). Moreover, given that the bioavailability of a powdered cherry capsule has not been explored previously, this might be responsible in part to the discrepancies reported in the literature.

The evidence surrounding the influence of MC ingestion on antioxidant status and oxidative stress is equally ambiguous. As previously highlighted, Bowtell et al. (2011) reported reductions in protein carbonyls with MC supplementation. Interestingly, this was observed despite a lack of improved total antioxidant capacity (TAC) following MC ingestion. In direct contrast, Howatson et al. (2010) reported that MC did not influence protein carbonyls, but was associated with increased total antioxidant status (TAS), as well as reduced uric acid and thiobarbituric acid reactive substances (TBARS). A study conducted by Bell, Walshe, et al. (2014) reported a reduction in concentrations of LOOH following repeated days of high intensity cycling when trained male cyclists were supplemented with 30 mL MC

concentrate twice per day for 7 days. However, more recently the research group demonstrated no change in LOOH with 8 days of MC supplementation following a single bout of high intensity cycling (Bell et al., 2015) and an adapted LIST protocol (Bell et al., 2016) compared to PL. It is likely that the differing exercise protocols (repeated vs single day) are once again largely responsible for these conflicting findings.

A number of research studies have demonstrated that MC ingestion attenuates declines in MVC (Bell et al., 2016; Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010), as well as cycling economy (Bell et al., 2015) and most recently, CMJ, 20 m sprint and 5-0-5 agility (Bell et al., 2016) following EIMD. It is likely that the antioxidant and anti-inflammatory properties of MC are able to protect against reductions in muscle functionality. However, this has been observed in the absence of an improvement in other measures of muscle function (Bell et al., 2015). The authors speculated that these differences might be explained by the muscular movements, complexity and skill involved in the assessment of muscle function. Given the participants were trained it is possible that a learning effect might be responsible for improved performance in some measures and not others (Bell et al., 2015). Nevertheless, all studies involving measures of muscle function have demonstrated that MC plays a role in attenuating the decline and accelerating the recovery of muscle functionality following EIMD.

To date, all studies examining the efficacy of MC on EIMD and recovery have adopted supplementation strategies which involve a preloading period, whereby MC is consumed prior to muscle-damaging exercise as well as during recovery. A number of studies have suggested that the preload supplementation may contribute to an enhanced antioxidant and anti-inflammatory status, as evidenced by increased antioxidant status (TAS) (Howatson et al., 2010), lower oxidative stress (LOOH) (Bell, Walshe, et al., 2014), and reduced inflammation (hsCRP) (Bell et al., 2015) prior to exercise. Moreover, evidence suggests that anthocyanins and other bioactive compounds have the potential to be stored. For instance, multiple doses of quercetin (a flavonoid metabolite which has a half-life of 11 to 28 h (Graefe et al., 2001; Hollman et al., 1997)) might result in plasma accumulation (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Manach et al., 2005). The persistent presence of anthocyanin metabolites in human excreta post consumption of anthocyanin rich

supplements (for instance in 24 h urine samples (Felgines et al., 2003) and 48 h faecal samples (Czank et al., 2013)) has also been suggested to indicate minor tissue accumulation (Kay, Mazza, Holub, & Wang, 2004). Interestingly, a recent study has demonstrated that a three-week supplementation of MC increased concentrations of a number of phenolic compounds in various tissues in a rodent model (Kirakosyan et al., 2015).

These data lend support to the notion that a preload of MC might play a role in the protection against initial muscle damage. For instance, a study has demonstrated a preservation of muscle function with MC immediately following exercise and throughout recovery with a 4-day MC preload (Bell et al., 2015). However, others have shown that the magnitude of the reduction in muscle function was similar immediately post exercise with an MC preload compared to PL (Bowtell et al., 2011; Howatson et al., 2010). Moreover, no study assessing systemic markers of myofibrillar disruption (CK and LDH) have reported an attenuated response following MC ingestion (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Bowtell et al., 2011; Howatson et al., 2010; Levers et al., 2015). Certainly, the bioactive components of MC do not provide rationale for the prevention of initial damage; rather it is the proposed reduction in oxidative stress and secondary muscle damage associated with inflammation (which may then attenuate further fibre disruption) that is thought to be responsible for accelerated recovery from EIMD. Moreover, it is important to note that some studies demonstrating differences between markers of inflammation and oxidative stress (Bell, Walshe, et al., 2014; Bell et al., 2015), and preservation of muscle function (Bell et al., 2015) with an MC preload have required participants to consume a low polyphenolic diet throughout trial periods. The reported differences between PL and MC groups in these studies may therefore be explained by a reduced antioxidant capacity in the PL group rather than an improvement elicited with MC supplementation; and this limits the application of the findings to true sporting scenarios.

Overall, it appears that there may be a beneficial role for MC in recovery from EIMD, particularly in regards to accelerated return of muscle function. Notably, no negative effects have been observed with MC supplementation. Efficacy for a preloading phase has been established in all studies and some evidence demonstrates enhanced antioxidant and anti-inflammatory status prior to exercise (Bell, Walshe,

et al., 2014; Bell et al., 2015; Howatson et al., 2010); perhaps attributable to tissue accumulation of MC polyphenolic compounds (Kay et al., 2004; Kirakosyan et al., 2015; Manach et al., 2004; Manach et al., 2005). While it has been suggested that this might reduce initial muscle damage, the current *status quo* maintains that it is primarily an attenuation of oxidative stress and the secondary inflammatory response that is responsible for ameliorations in recovery from EIMD. Yet, the proposed mechanisms of action in regards to the accelerated recovery following MC supplementation are somewhat inconsistent; with reports of improved antioxidant status and/or reduced oxidative stress in the absence of reductions in inflammatory markers (Bowtell et al., 2011) and vice versa (Bell et al., 2016; Bell et al., 2015). The range of participant demographics, dietary control procedures, and exercise protocols employed clearly contributes to the equivocal findings. Indeed, only one study has investigated the effects of MC following a repeated-sprint exercise paradigm (Bell et al., 2016). Additionally, whilst females have been included in mixed-sex populations (Howatson et al., 2010; Kuehl et al., 2010), there are potential sex differences in EIMD and recovery (Kendall & Eston, 2002). Moreover, given their structural similarities to oestrogen, polyphenolic secondary plant metabolites (including flavones, flavonols and isoflavones for example) appear to exert oestrogenic effects (Miksicek, 1995), and thus modulate and affect the bioavailability of endogenous oestrogens (Ward & Kuhnle, 2010). As such, since oestrogen is thought to play a key role in the observed sex differences in EIMD, currently the complete lack of studies investigating the supplementation of MC in a female only population is surprising and warrants research.

2.3.2 Whey protein hydrolysate

2.3.2.1 Background

Protein metabolism represents the constant regulation of protein synthesis and protein breakdown through various metabolic processes in the body. Changes in the magnitude and duration of these periods of synthesis and breakdown determine net protein balance; either positive or negative (Tipton, 2008). Measuring changes in whole-body and muscle protein balance is challenging; not least because protein synthesis and breakdown *in vivo* are not constant processes and as a result both

arterial and intracellular amino acid concentrations fluctuate continuously (Tipton, 2008). Indeed, due to its constant state of flux, it has been suggested that the transient changes in muscle protein metabolism may only be detected with minute-by-minute biopsy samples (Tipton, 2008). While advances in measurement techniques *in vivo* (including stable isotopic labelling and gene expression) allow for relatively reliable measures of muscle protein synthesis (MPS) and measures of muscle protein breakdown (MPB), these are more difficult to measure during exercise, mostly due to changes in tracer uptake and release from muscle as a consequence of changes in blood flow (Kumar, Atherton, Smith, & Rennie, 2009). Nevertheless, protein balance appears to be largely affected by both resistance and non-resistance type exercise (for a detailed overview, the reader is directed to previously published critical reviews (Kumar et al., 2009; Rennie & Tipton, 2000)). During exercise it appears that MPS is reduced (Bowtell et al., 1998; Dreyer et al., 2006; Rennie et al., 1980), whereas MPB remains unchanged from rest (Durham et al., 2004; Tipton et al., 2001). However, the increased requirement for protein post exercise stimulates MPS, potentially at the myofibrillar, sarcoplasmic, and mitochondrial levels (Tipton, 2008), which has been shown to persist for 48 h (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997) following resistance exercise and up to 72 h in non-resistance type exercise in the fed state (Miller et al., 2005). While this has been suggested to be unrelated to muscle contraction performed (Phillips et al., 1997), others suggest that MPS appears to be greater following damaging eccentric contractions compared to concentric contractions (Eliasson et al., 2006; Moore, Phillips, Babraj, Smith, & Rennie, 2005); perhaps mediated through a combination of greater tension and stretching of the muscle (Eliasson et al., 2006). Proteolysis is also elevated post exercise, although relatively short-lived (several hours (Rennie & Tipton, 2000)) compared to synthesis which persists for much longer. Indeed in response to exercise and feeding, a five-fold difference in MPS relative to MPB has been reported (Rennie & Wilkes, 2005).

The repair and reconditioning of skeletal muscle, which is vital for maximising recovery requires MPS to exceed MPB (Hawley, Tipton, & Millard-Stafford, 2006; Saunders, 2007; Tipton, 2008; Tipton & Wolfe, 2001). Despite an increase in MPS, at least in the fasted state (and although the associated rise in insulin with exercise diminishes the catabolism of protein (Beelen, Burke, Gibala, & van Loon, 2010;

Tipton, 2008)) there is negative net muscle protein balance post exercise which only becomes positive through an exogenous provision of amino acids (Kumar et al., 2009; Phillips et al., 1997; Pitkanen et al., 2003). Conceivably, protein intake may provide the abundant availability of amino acids necessary for improving protein balance. In addition, elevated blood flow, which accompanies exercise, increases the transport of amino acids to the muscle. This may explain, at least in part, the additive effect of the combination of protein and/or amino acid administration and resistance (Børsheim, Tipton, Wolf, & Wolfe, 2002; Koopman et al., 2005; Levenhagen et al., 2002; Tipton et al., 2007), and non-resistance type exercise (Donges et al., 2012; Gibala, 2007; Howarth, Moreau, Phillips, & Gibala, 2007, 2009; Levenhagen et al., 2002) on improving muscle protein balance.

This improved muscle protein balance with protein ingestion following exercise is the proposed mechanism of action for its mitigating effects on EIMD. However, the evidence in the literature does not wholly support this idea and the effects of protein and amino acid sources on muscle damage and recovery are unclear. Certainly, while a number of research studies have examined the role of protein supplementation strategies in preventing or alleviating the symptoms associated with EIMD, for the most part much of the evidence is equivocal. Inconsistencies in the literature can be predominantly explained by differences in experimental designs, namely variances in study populations (age, sex, and training status) and the supplement intervention implemented (form, dose and frequency). For instance, since protein sources differ in their amino acid profiles they have different digestive properties. The digestion and absorption rates determine the subsequent appearance of amino acids in plasma (Koopman et al., 2009) and their availability for protein synthesis. Therefore, the type of protein is an important consideration when evaluating the EIMD literature.

The milk-derived proteins casein and whey, are generally considered to have the greatest bioavailability when compared to other protein substrates (Campbell et al., 2007). Whey protein forms about 20% of protein in whole bovine milk and induces a faster postprandial rise in amino acid availability than casein due to its water solubility and rapid digestion and absorption (Boirie et al., 1997; Dangin et al., 2001). Moreover, whey contains a higher abundance of branched-chain amino acids (BCAA) and in particular higher leucine concentrations (Tang & Phillips, 2009).

Leucine is thought to induce the most potent effect in regulating protein metabolism among the BCAA (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004) and plays an important role in MPS following exercise (Norton & Layman, 2006). Therefore, in terms of practical application, whey is thought to be most suited for post exercise consumption. Whey protein is commercially available as whey protein concentrate (WPC; or native whey) or whey protein isolate (WPI; containing more essential amino acids and BCAA and may be particularly appealing for lactose intolerant individuals due to little lactose content). Moreover, these proteins can be hydrolysed; a process which partially breaks down and pre-digests the protein when exposed to heat, enzymes, or acids and produces large quantities of shorter peptide chains. As such it is recognised that protein hydrolysates are more readily digested and absorbed, and increase circulating amino acid concentrations more rapidly than intact proteins (Koopman et al., 2009; Manninen, 2004; Morifuji et al., 2010; Silk et al., 1979) as illustrated in Figure 5. Indeed, protein hydrolysates elicit a greater insulin response (Manninen, 2006; Power, Hallihan, & Jakeman, 2009); potentially enhancing protein anabolism (Calbet & MacLean, 2002; Manninen, 2009). Ultimately, a rapid delivery of free amino acids to skeletal muscle with hydrolysed protein may improve protein balance and accelerate recovery following damaging exercise. However, few research studies have been conducted to investigate the influence of whey protein hydrolysate (WPH) on recovery following muscle-damaging exercise (summarised in Table 3).

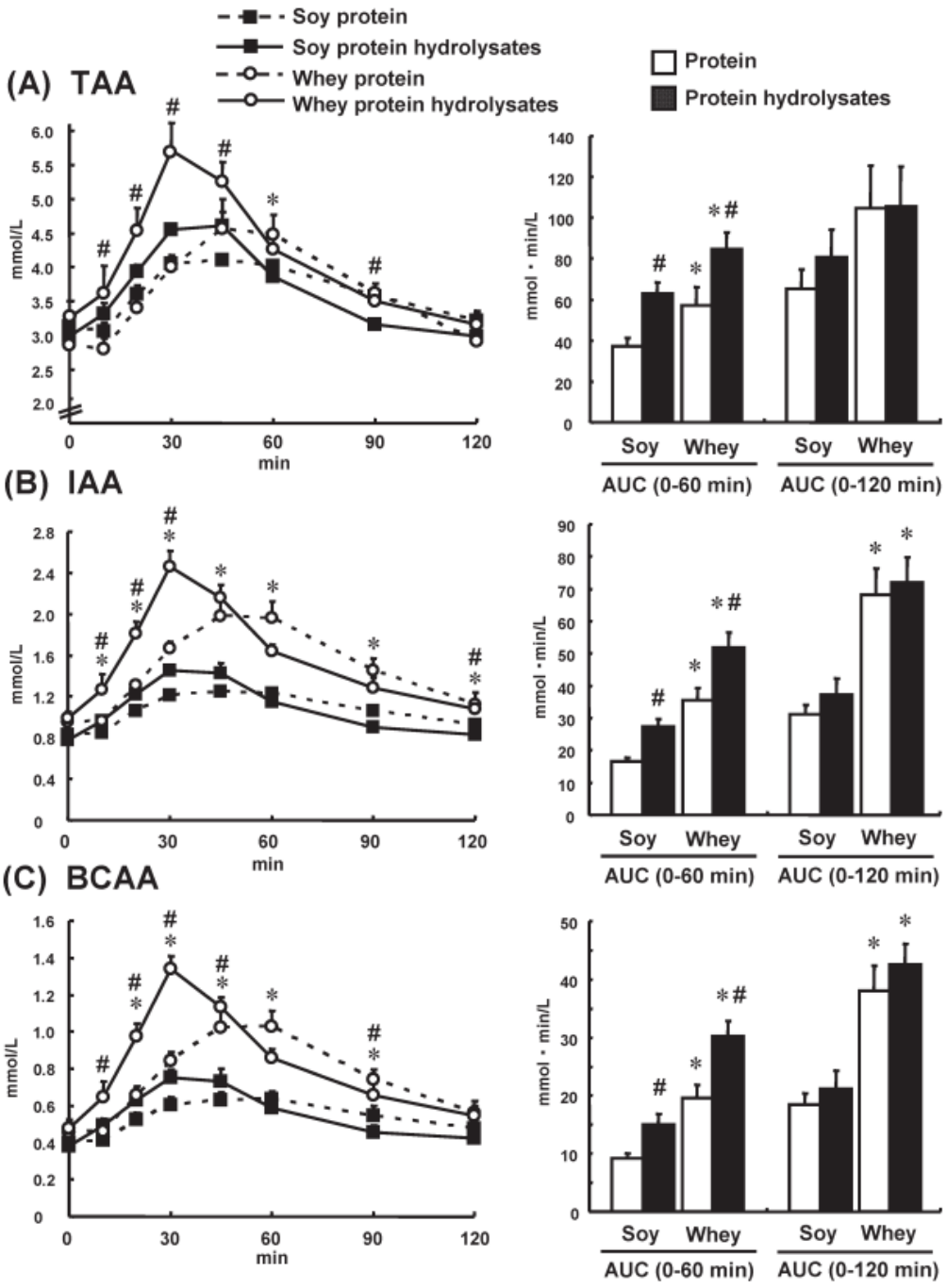

Figure 5. Plasma concentrations of (A) total amino acids, (B) indispensable amino acids, and (C) branched-chain amino acids (Morifuji et al., 2010).

*Graphs on the left illustrate plasma concentrations over 120 min period, and graphs on the right illustrate the area under the curve for the 0-60 and 0-120 min period. Values are presented as means ± SEM, significance at $p < 0.05$, $n = 5$ per group. #denotes significant difference between protein hydrolysates and non-hydrolysed protein. *denotes significance between dietary protein source.*

Table 3. WPH effects on recovery from EIMD.

				Supplement		Measure of EIMD		
Author	**Subjects**	**Design**	**Exercise**	**Dosage**	**Duration**	**Marker**	**Time points**	**Effect of WPH**
Hansen et al., 2015	10 female 8 male elite orienteers (WPH-CHO group, 4M [23.8 ± 2.8 y] and 5F [21.2 ± 3.1 y]; CHO group, 4M [21.2 ± 1.7 y] and 5F [20.0 ± 2.1 y])	Randomised, counter-balanced, single-blind. Dietary control throughout. (WPH-CHO, n=9 vs isocaloric CHO, n=9)	1 week training camp (13 sessions)	0.3 g·kg^{-1} WPH pre and 0.3 g·kg^{-1} WPH with 1 g·kg^{-1} CHO post each exercise session	7 days	CK, LDH, myoglobin, IL-6, GM-CSF, IFN-c, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, cortisol, immunoglobulin A, performance capacity and motivation, 4 km performance (time trial)	Days 1, 3, 5, 6 and 7 and post 4km run (blood and saliva only). Performance capacity and motivation measured twice per day during training camp (morning and after last exercise session each day). 4km performance pre and post training camp	↓CK ↑sense of performance capacity, 4km performance
Rahbek et al., 2015	24 recreationally active males (23 y [95% CI 21.1; 23.9])	Randomised, double-blind, placebo-controlled (WPH-CHO, *n*=12 vs isocaloric CHO, *n*=12)	150 eccentric contractions of knee extensor	28 g WPH with 28 g CHO, 3 times per day (0, 3, 6 h post measures)	3 days (day of exercise and 2 days of recovery)	DOMS, Akt-mTOR and FOXO signally proteins, CK, MVC	Pre, (and additional 3 h biopsy), 24, 48, (and additional 72, 96 and 168 h post exercise for DOMS, CK and MVC)	↓ p-Akt in exercised leg ↑ p-FOXO1 in control leg and DOMS
Farup et al., 2014	24 recreationally active males (WPH-CHO 22.5 y [95% CI 21.1; 23.9]; PL 24 y [95% CI 22.3; 25.7])					DOMS, muscle SC content, CK, MVC	Pre, (and additional 3 h biopsy), 24, 48, (and additional 72 and 96 h for DOMS, CK and MVC) and 168 h post exercise	↑SC proliferation and DOMS

Table 3. Continued

				Supplement		Measure of EIMD		
Author	**Subjects**	**Design**	**Exercise**	**Dosage**	**Duration**	**Marker**	**Time points**	**Effect of WPH**
Lollo et al., 2014	24 professional male soccer players (18 ± 0.8 SEM y)	Double-blind, placebo-controlled. Dietary control throughout. (WPH, *n*=8 vs isocaloric CHO, *n*=8 vs isoproteic WPC, *n*=8)	12 week training program	0.5 $g \cdot kg^{-1}$, twice per day (pre and post training)	12 weeks	CK, LDH, uric acid, creatinine, yo-yo test, 4 min time trial, SJ, CMJ, CMJ with hands	Pre and post 12 week training program	↓CK, LDH, and 4 min time trial
Buckely et al., 2010	28 sedentary males (18-30 y)	Randomised, double-blind, placebo-controlled (WPH, *n*=6 vs non-caloric PL, *n*=11 vs isoproteic WPI, *n*=11)	100 eccentric contractions of knee extensor	250 mL flavoured water (PL) with 25 g, three times (0, 6, and 22 h post exercise)	1 day	DOMS, CK, TNF-α, MVC	Pre and 0, 1, 2, 6 and 24 h post exercise	↑MVC
Cooke et al., 2010	17 untrained males (23 ± 5 y)	Randomised, double-blind, placebo-controlled (WPH-CHO, *n*=9 vs isocaloric CHO, *n*=8)	40 x leg press, leg extension, and leg curl at 120% 1RM	1.5 $g \cdot kg^{-1} \cdot day^{-1}$ WPH in 8% CHO solution. Within 30 mins on exercise day, every other day divided in 4 portions throughout the day	14 days (day of exercise, and 1,2,3,4,710 and 14 days post)	CK, LDH, isokinetic and isometric strength	Pre (and additional 0.5, 1, 2 and 4 h for blood indices only), and 1, 2, 3, 4, 7, 10 and 14 days post exercise for blood and muscle function	↑isometric force

EIMD, exercise-induced muscle damage; CI, confidence interval; SEM, standard error of the mean; M, male; F, female; PL, placebo; WPH, whey protein hydrolysate; WPC, whey protein concentrate; WPI, whey protein isolate; CHO, carbohydrate; WPH-CHO, combined WPH and CHO; 1RM, one-repetition maximum; DOMS, delayed-onset muscle soreness; CK, creatine kinase; LDH, lactate dehydrogenase; IL, interleukin; TNF-α, tumor necrosis factor alpha; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-c, interferon-gamma; mTOR, mechanistic target of rapamycin; Akt, protein kinase B; p-Akt, phosphorylation of Akt; FOXO, fork head box transcription factors; p-FOXO1, phosphorylation of FOXO class O1; SC, satellite cell; CMJ, countermovement jump; SJ, squat jump; MVC, maximum voluntary contraction.

2.3.2.2 Evidence for the efficacy of whey protein hydrolysate for recovery

Most recently, Hansen, Bangsbo, Jensen, Bibby, and Madsen (2015) aimed to investigate the effect of consuming 0.3 $g \cdot kg^{-1}$ of WPH prior to and 0.3 $g \cdot kg^{-1}$ of WPH with 1 $g \cdot kg^{-1}$ carbohydrate (CHO) following a number of exercise sessions during a 7-day training camp in male and female elite orienteers. Compared to isocaloric CHO supplementation, concentrations of CK were attenuated on days 3, 5, 6 and 7 of the training camp with WPH and CHO supplementation (WPH-CHO). Moreover, decreases in psychological sense of performance capacity throughout the training camp were attenuated, and 4 km time trial performance was improved following the intervention with WPH-CHO compared to isocaloric CHO supplementation. However, interestingly, myoglobin, LDH, plasma cortisol, salivary immunoglobulin A, and a number of plasma cytokine concentrations were not different between groups. This suggests that improved recovery with WPH may not be attributable to reductions in the inflammatory response. A notable strength of this study was the dietary control employed throughout the testing period. The macronutrient composition was similar between groups (15, 63 and 22% of energy intake derived from protein, carbohydrate and fat respectively) and specifically, participants were provided with quantities of protein (> 1.7 $g \cdot kg^{-1} \cdot day^{-1}$) and carbohydrate (> 8 $g \cdot kg^{-1} \cdot day^{-1}$) above recommended intakes for elite endurance athletes (Rodriguez, Di Marco, & Langley, 2009). Therefore, given that muscle glycogen restoration and protein synthesis are expected to be maximised with these intakes, the observed improvements with WPH-CHO are likely to be as a result of the added protein in this group.

Similarly, Lollo et al. (2014) reported beneficial effects of consuming WPH for recovery from muscle-damaging exercise despite participants achieving the 1.2-1.7 $g \cdot kg^{-1} \cdot day^{-1}$ protein recommended for athletic populations (Tipton & Wolfe, 2004). The controlled diet (consisting of 1.3 $g \cdot kg^{-1} \cdot day^{-1}$ protein) of professional male soccer players was supplemented with an additional 1 $g \cdot kg^{-1} \cdot day^{-1}$ of WPH in two equal portions during a 12-week training program. Compared to pre-supplementation, CK and LDH concentrations following the 12-week supplementation period were attenuated by 42 and 30%, respectively with WPH, while concentrations following isoproteic WPC and isocaloric CHO

supplementation remained unchanged (Lollo et al., 2014). However, in contrast to Hansen et al. (2015) these reductions did not elicit improvements in muscle function as measured by SJ and CMJ height, the yo-yo test, and 4 min time trial performance (which was improved with isocaloric CHO only). Nevertheless, these long-term supplementation strategies provide evidence that WPH might effectively attenuate symptoms associated with EIMD induced by repeated exercise sessions during a training program. Since regular intake of protein is suggested to maintain an anabolic state and maximise synthesis rates (Beelen et al., 2010), it is perhaps unsurprising that these studies demonstrated beneficial effects over a prolonged supplementation period. In addition, these findings are in agreement with a number of studies showing efficacy of additional protein supplementation for exercise recovery in spite of participants consuming protein intakes beyond recommended levels (Coombes & McNaughton, 2000; Jackman, Witard, Jeukendrup, & Tipton, 2010).

While Hansen et al. (2015) and Lollo et al. (2014) demonstrated that WPH ingestion effectively attenuated CK, some studies have found no differences in the CK response following EIMD with WPH-CHO compared to isocaloric CHO (Cooke et al., 2010; Farup et al., 2014; Rahbek, Farup, de Paoli, & Vissing, 2015), or WPH alone compared to intact whey protein, and non-caloric PL (Buckley et al., 2010). However, in the absence of reductions in CK and LDH, a 14-day supplementation of 1.5 $g \cdot kg^{-1} \cdot day^{-1}$ WPH attenuated isometric force loss 3 and 7 days following an acute resistance exercise bout in untrained males (Cooke et al., 2010). Similarly, an acute supplementation study (Buckley et al., 2010) reported an improved recovery of peak isometric torque from eccentric EIMD with 75 g WPH versus its WPI substrate source in untrained males (observed by 6 h post exercise having consumed 25 g), despite no reduction in circulating concentrations of CK or TNF-α. It is important to note that these systemic markers were unchanged following the eccentric exercise protocol in all groups (Buckley et al., 2010); suggesting that the stimulus was not successful at eliciting a large muscle-damage response. However, given that there was an approximate 23% reduction in peak isometric torque post EIMD, it is likely that the acute recovery period chosen to measure dependent variables (< 24 h) was too short for changes in systemic markers to manifest; thus making inference beyond this time point difficult. Nonetheless, it is not clear

whether improvements in muscle functionality with WPH are as a result of attenuated myofibrillar disruption.

Alternative mechanisms for the mitigating effects of WPH supplementation against EIMD have been explored previously. Farup et al. (2014) investigated the effect of WPH supplementation on fiber type-specific satellite cell (SC) accumulation following eccentric exercise. The authors reported that, compared to isocaloric CHO, 84 $g \cdot day^{-1}$ WPH with 84 $g \cdot day^{-1}$ CHO for three days accelerated the increase of mixed fibre SC proliferation (notably in type II fibres) observed in muscle biopsy samples following an acute eccentric exercise bout in recreationally active males. Though providing potential support for the repair and remodelling of muscle fibres following high-intensity eccentric exercise, this was not reflected in differences in muscle function (MVC) or circulating CK compared to an isocaloric CHO supplement. In addition, the WPH group experienced greater muscle soreness 96 h post exercise compared to isocaloric CHO (Farup et al., 2014). A similar study (Rahbek et al., 2015) conducted by the same research group reported an increase in phosphorylation of mechanistic target of rapamycin (mTOR), ribosomal protein S6 kinase beta-1 (p70S6K) and ribosomal protein S6 (rpS6), and a decrease in phosphorylation of forkhead box O1 (FOXO1) and forkhead box O3 (FOXO3) in an eccentrically induced muscle damaged leg, with no group differences between supplements. However, interaction effects demonstrated that phosphorylation of Akt kinase was lower in the exercised leg, and phosphorylation of FOXO1 was higher in the control leg following WPH-CHO compared to isocaloric CHO. Yet, again these changes in signalling pathways were not correlated with rate of muscle force recovery. Despite these studies failing to observe improvements in muscle function, the myocellular effects that were reported are nevertheless thought to contribute to the repair of damaged muscle and could represent potential mechanisms responsible for the role of WPH in accelerating recovery from EIMD.

Interestingly, few studies have measured the effect of WPH on subjective muscle soreness, and no differences (Buckley et al., 2010), or indeed detrimental effects (Farup et al., 2014; Rahbek et al., 2015) have been reported. This is surprising given that the increased muscle soreness with WPH did not result in concomitant decreases in MVC (Farup et al., 2014; Rahbek et al., 2015), and in fact MVC was re-established above pre-exercise levels by 6 h post exercise with WPH in the

absence of group differences in muscle soreness (Buckley et al., 2010). Kirby et al. (2012) has previously demonstrated that leucine supplementation elicited increases in muscle soreness compared to a PL post EIMD; however, the authors could not explain this unexpected result. Certainly, the lack of a beneficial treatment effect in muscle soreness in the WPH literature is in contrast to a number of studies investigating the efficacy of protein supplementation in attenuating EIMD (Howatson, Hoad, et al., 2012; Jackman et al., 2010; Shimomura et al., 2010). It has been suggested that increased total energy intake provided by the additional protein supplementation in these studies is responsible for the decrease in muscle soreness (Jackman et al., 2010). Although it is thought that amino acid availability is more important than energy availability for post exercise protein synthesis (Levenhagen et al., 2002) and accelerated recovery from EIMD, additional calories cannot be discounted as contributing (at least in part) to observed recovery benefits. Indeed, the studies investigating WPH which failed to report treatment effects for muscle soreness included isoproteic WPI (Buckley et al., 2010) or isocaloric CHO (Farup et al., 2014; Rahbek et al., 2015) controls for comparison. In addition to the difference in type of protein supplements investigated in the literature, the notable variance in the comparable supplement is also largely responsible for the inconsistencies of documented findings in this area. Therefore, the role of protein in reducing muscle soreness, and the potential mechanism responsible for this is not clearly indicated.

To conclude, the evidence for WPH in isolation or in combination with CHO on reducing markers of muscle damage and accelerating recovery are encouraging, particularly as improvements have been demonstrated in recreationally active (Farup et al., 2014; Rahbek et al., 2015) and highly trained individuals (Hansen et al., 2015; Lollo et al., 2014). Though inconclusive (and inconsistent amongst indirect markers of damage), these data appear to suggest potential superiority of WPH over other forms of whey (Buckley et al., 2010; Lollo et al., 2014) and isocaloric CHO (Cooke et al., 2010; Farup et al., 2014; Hansen et al., 2015; Lollo et al., 2014; Rahbek et al., 2015) supplements for EIMD. This has been observed with both long-term (Cooke et al., 2010; Hansen et al., 2015; Lollo et al., 2014) and more acute (Buckley et al., 2010; Farup et al., 2014; Rahbek et al., 2015) supplementation strategies; with even a single 25 g dose eliciting beneficial effects for muscle function within 6 h post EIMD (Buckley et al., 2010). In addition, the ability to attribute treatment effects to

the WPH is amplified given that all studies have employed the use of an isocaloric treatment group for comparison. However, presently, the efficacy of WPH in accelerating recovery from EIMD has been investigated following acute eccentric/resistance exercise bouts (Buckley et al., 2010; Cooke et al., 2010; Farup et al., 2014; Rahbek et al., 2015) or longer-term training programmes (Hansen et al., 2015; Lollo et al., 2014), and no study has examined effects following an acute bout of repeated-sprint exercise. Moreover, all investigations exploring the influence of WPH on EIMD and recovery have been conducted with male or mixed sex groups. Given the potential sex differences in EIMD previously discussed, this is an important limitation in the literature examining the efficacy of WPH for accelerating recovery from EIMD.

2.3.3 Summary

Currently the evidence surrounding the efficacy of MC and WPH for attenuating EIMD and ameliorating recovery is promising. Certainly, no studies have observed adverse effects of MC. While an increase in muscle soreness at one time-point (Farup et al., 2014; Rahbek et al., 2015) and a decrease in 4 min time trial (Lollo et al., 2014) post EIMD has been reported with WPH compared to PL, these findings were observed alongside beneficial effects. To date, only two studies investigating supplementation of MC (Howatson et al., 2010; Kuehl et al., 2010) and only one study investigating supplementation of WPH has included female participants (Hansen et al., 2015); and while the distribution of men and women were relatively balanced between groups, these authors failed to acknowledge the influence of menstrual cycle phase and contraceptive use on EIMD and recovery. Moreover, studies examining WPH have either employed an acute eccentric exercise bout (Buckley et al., 2010; Cooke et al., 2010; Farup et al., 2014; Rahbek et al., 2015) or a number of exercise sessions over a training program (Hansen et al., 2015; Lollo et al., 2014) to induce muscle damage. Similarly, only one study has investigated the influence of MC supplementation following a repeated-sprint exercise (Bell et al., 2016). There is a growing body of evidence surrounding the efficacy of MC and WPH for attenuating muscle damage and accelerating recovery. However, further research is required; certainly, in females and following different exercise

paradigms, where evidence is lacking. These limitations in the literature provide rationale for the experimental work detailed later in the thesis.

3 Energy intake and energy expenditure of pre-professional female contemporary dancers in full-time dance training.

Publication arising from this chapter:

Brown, M. A., Howatson, G., Quin, E., Redding, E., & Stevenson, E. J. (2017). Energy intake and energy expenditure of pre-professional female contemporary dancers. *PLoS One*, *12*(2).

3.1 Introduction

Dance is characterised as a moderate-high intensity, high skill, and predominantly intermittent activity (Beck, Redding, et al., 2015). These characteristics can vary, largely dependent on the style of dance and the capacity in which it is performed. The daily training schedule of a dancer is difficult to define but typically includes multiple training sessions, consisting of technique classes, rehearsals, and/or performances. The intensity and volume of exercise previously reported (Twitchett et al., 2010) can often be comparable to that of many elite athletes. For instance, workloads of professional dancers may involve 6-10 h of dance training per day (Wyon, 2010) and this may be accompanied by additional fitness training. The workloads of trainee dancers as part of dance school programs are also reported to be high, particularly prior to studio showings and exams (Grove et al., 2013). In addition, since dance is principally an art form, it demands artistry and expression as well as physical and technical skill. As with many comparable aesthetic sports, while extremely low body mass and fat mass are known to negatively influence performance and recovery potential, low levels are nevertheless often considered to be advantageous for movement efficacy and artistic expression (Sundgot-Borgen & Garthe, 2011). Indeed, maintaining a lean physique is thought to be an important aspect of dance fitness and a pre-requisite for success in the profession (Claessens et al., 1987; Hergenroeder et al., 1993). As a result, a dichotomous issue arises in dance, whereby attaining the desired body composition can be a conflicting component in the pursuit of optimal performance and recovery.

The typical energy intake and energy expenditure of athletes has been explored in a number of sports, for instance in football (Briggs, Cockburn, et al., 2015; Russell & Pennock, 2011), taekwondo (Cho, 2014), and gymnastics (Silva & Paiva, 2014). However little is known about the nutritional and energy intakes of a dance population. This is surprising given that many athletes in aesthetic or weight dependent sports fail to compensate high energy demands with an adequate energy intake, and are at risk of numerous health and performance impairments associated with energy imbalance (Loucks, 2004). For instance, potential issues arising from inadequate nutrition in dancers include insufficient peak bone mass and menstrual dysfunction (Kaufman et al., 2002; Warren, Brooks-Gunn, et al., 2002). A recent

review (Beck, Redding, et al., 2015) has summarised the research investigating the energy demands of dance; largely through measurement of heart rate and oxygen cost. Though the authors conclude that the majority of investigations describe the energy demand to be moderate-high and intermittent, they noted a number of methodological limitations. Additionally, while these studies have identified energy demands in regards to a single movement, class, or performance, few have investigated these in nutritional contexts (i.e. kcal) or investigated the longer-term energy demands. Similarly, while a number of studies have sought to identify the dietary intakes of dancers, only a handful have looked at this in parallel with their physical activity or energy expenditure (Beck, Mitchell, et al., 2015; Burckhardt et al., 2011; Dahlstrom et al., 1990; Doyle-Lucas et al., 2010; Frusztajer et al., 1990; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002). The majority of these studies determined that dancers were (for the most part) in negative energy balance or very low energy availability (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Doyle-Lucas et al., 2010; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002). However, these investigations used a range of measurement techniques to determine TEI and TEE, and whilst the methods previously employed provide an indication of energy balance, their validity has been questioned; thus limiting the strength of their findings. For instance, heart rate monitoring is not a reliable indicator for estimations of energy demand given the intermittent nature of dance (Redding et al., 2004), and self-reported dietary records are limited by under/over eating and/or reporting (Magkos & Yannakoulia, 2003). Indeed, a study in female ballet dancers reported a mean bias to under-reporting of 667 kcal·day^{-1} or 21% of energy intake when comparing 4-day weighed food recording and energy expenditure via doubly labelled water (Hill & Davies, 1999).

Although the low body mass index (BMI) and body fat levels frequently reported in dancers (Calabrese et al., 1983; Cohen et al., 1985; Hamilton et al., 1988; Laws, 2005; van Marken Lichtenbelt et al., 1995) suggest that exercise and/or eating behaviours may be suboptimal, the inherent limitations in study designs render previous conclusions of poor nutritional intake and energy balance questionable.

Moreover, the majority of research has been conducted in ballet and little has been published in modern/contemporary equivalents. Research has demonstrated differences in artistic and physical demands of ballet and contemporary styles (Wyon et al., 2011) and body composition data reveal that ballet dancers tend to be the leanest (Liiv et al., 2013; Pacy et al., 1996). Thus, it is not appropriate to assume that the literature regarding one dance style is relevant and directly transferable to the other.

Whilst aesthetics are important, dancers are subject to similar physiological stressors as other athletes, and dietary habits can affect both dance performance (Sandri, 1993) and certainly exercise recovery (Beelen et al., 2010). The latter is particularly important given that dancers are expected to maintain consistently high levels of performance and to perform on demand; yet the aforementioned training and performance schedules of both professional and pre-professional dancers demonstrate that recovery periods are often short. Indeed, in dance populations, optimal recovery and maintaining an ability to perform on a daily basis is often the primary goal. Consequently, dancers would benefit from a greater understanding of their energy requirements to support their training schedules; not least to ensure optimal performance, but also to maximise recovery. Therefore, this investigation sought to determine the energy and macronutrient intake and energy expenditure of pre-professional female contemporary dancers during a 7-day period of full-time training at a conservatoire A secondary objective was to compare exercise and dietary behaviours during week days (Monday-Friday; where there was scheduled dance training), and during the weekend (Saturday and Sunday; where there was no scheduled dance training). Accordingly, this chapter sought to address the first aim of the thesis: '*to determine the typical training and eating behaviours of pre-professional female dancers*'.

3.2 Materials and methods

3.2.1 Participants

Twenty-five pre-professional female undergraduate contemporary dance students attending a conservatoire volunteered for the study (mean ± SD age 21 ± 2 y; stature 167.4 ± 5.9 cm; mass 63.4 ± 6.9 kg; and BMI 22.6 ± 2.0 $kg \cdot m^{-2}$) and written informed consent obtained (Appendix A). The sample size was determined by completing a power analysis (power = 0.8, $\alpha = 0.05$) based on energy balance data from Kostrzewa-Tarnowska & Jeszka (2003). This determined a sample size of nine would provide statistical power above 80%, with an alpha level of 0.05. This observational, cross-sectional study aimed to recruit as many participants as feasible within time and logistical constraints. Exclusion criteria were pregnancy, and presence of any medical or physical conditions, either chronic or sustained in the preceding 3 months which would make participation difficult or harmful to the participant. Contraceptive use (regardless of form) was not an exclusion criterion. Participants were free from injury and were participating fully in all scheduled dance training ($n = 14$, $n = 8$ and $n = 3$ in 1st, 2nd and 3rd years, respectively of a three-year full-time undergraduate BA Contemporary Dance degree). All participants were instructed to maintain their typical dietary and physical activity behaviours throughout data collection. The study was conducted in the weeks before the end of the academic year (during May-June), when students were participating in normal training as well as rehearsing for end of year productions. The study was conducted according to the guidelines of the Declaration of Helsinki and all experimental procedures were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria (HLSMB240215). Institutional Approval was also obtained from Trinity Laban Conservatoire of Music and Dance.

3.2.2 Questionnaires

Participants completed the Healthier Dance Practice National Survey (also referred to as the Fit to Dance 2 national survey; Appendix B) (Laws, 2005) to provide a range of information including dance background as well as dietary history. A

menstrual cycle questionnaire (Appendix C) was completed to assess menstrual cycle history and, where possible, to identify menstrual cycle phase. In addition, an 18 item, 3-Factor Eating Questionnaire (TFEQ-R18; Appendix D) (Karlsson, Persson, Sjostrom, & Sullivan, 2000) was used to assess three eating behaviours; restrained eating (conscious restriction of food intake in order to control body weight or to promote weight loss), uncontrolled eating (tendency to eat more than usual due to a loss of control over intake accompanied by subjective feelings of hunger), and emotional eating (inability to resist emotional cues). The degree of expression (0-100%) of each eating behaviour was determined by comparing absolute scores relative to the proportion of the highest possible scores; with higher values indicating more of the behaviour (Anglé et al., 2009). The TFEQ-R18 has demonstrated adequate internal consistency reliability coefficients for each of the three subscales (Cronbach's $\alpha > 0.70$) (de Lauzon et al., 2004; Karlsson et al., 2000). All data from questionnaires were anonymised.

3.2.3 Body composition

Stature was measured to the nearest 1 mm (stretch stature technique, Model 220, Seca Ltd, Birmingham, UK) and body mass to the nearest 0.1 kg (Model 876, Seca Ltd, Birmingham, UK). As per the standard techniques of the International Society for the Advancement of Kinanthropometry (ISAK) (Marfell-Jones, Olds, & Stewart, 2011), calibrated Harpenden calipers (CMS Weighing Equipment Ltd, London, UK) were used for skinfold measurement at 7 sites (biceps, triceps, subscapular, suprailiac, abdominal, front thigh and medial calf). An anthropometric tape measure (Luftkin, Maryland, USA) was used for land marking and measuring waist and hip circumference to determine waist to hip ratio (W:H). All measurements were taken on the right side of the body and measured in duplicate. When differences in skinfold and circumference were greater than 0.5 mm and 5 mm, respectively, additional measures were taken, and the mean of two measures within this range were used for analysis. All measurements were taken by the principal investigator who demonstrated good intra-rater reliability (technical error of measurement (TEM) < 4% and < 2% for skinfold and circumference, respectively), and good inter-rater reliability when compared to a Level 2 ISAK accredited researcher (TEM

of < 10% and < 2% for skinfold and circumference, respectively). Percentage body fat (%BF) was estimated using the Siri equation (Siri, 1961) following determination of predicted body density from the sum of 4 skinfolds (biceps, triceps, subscapular and suprailliac) (Durnin & Womersley, 1974). This then allowed for estimation of fat and fat free mass (FFM).

3.2.4 Energy intake

Participants were asked to complete a 7-day weighed food diary to provide a detailed description of their food and fluid intake. Participants were given comprehensive verbal and written instructions to familiarise them with this data collection method prior to testing, including an example diary entry in order to demonstrate the level of detail required. Participants were instructed to report time of consumption, how food/fluid was cooked or prepared, brand names, and quantities; electronic portable scales were provided. Where applicable, recipes were requested, as well as information regarding supplement use. Where weighing was not possible, participants were asked to provide information regarding portion size comparable to household measures (for example cup, teaspoon, and tablespoon) and these were clarified with photographic evidence to improve the estimation of intakes.

In addition each participant engaged in a 24-hour recall interview using the two-pass method (Ashley & Bovee, 2007) on each day of the data collection period to be cross-referenced with the food diary. This allowed the researcher to clarify ambiguous information and complete diary entries with missing data. Where possible these interviews were conducted face-to-face, however for logistical reasons 11 of 175 (6%) were conducted over the phone. In this case, participants were instructed to recall food and fluid intake from memory in a quiet space as normal; without reference to their food diary. The combined method of self-reported weighed food diary and 24-hour recall interview has been found to result in good agreement with the gold standard observed food intake technique (Briggs, Rumbold, et al., 2015; Rumbold et al., 2011).

Commercially available dietary analysis software (Nutritics Ltd V4, Swords, Ireland) was used to calculate total energy intake (TEI). A single researcher

(principal researcher) analysed the dietary data in order to avoid variability in interpretation of these data and enhance reliability (Deakin, 2000). Where foods were not listed in the dietary database, the product label was consulted and the energy and macronutrient (and where possible micronutrient) composition entered manually. A 7-day period is thought to best represent the variety of dietary and physical activity practices and are associated with the most valid nutritional information (Bingham, 1987). All testing was conducted in free-living conditions and no attempts were made to influence the diet of participants.

3.2.5 Energy expenditure

A tri-axial accelerometer (ActiGraph GT3X+, Pensacola, Florida, USA) was secured under clothing with an elastic belt on the right hip. This was worn continuously (except during activities which would submerge the accelerometer in water) throughout the same 7-day period that was analysed for dietary intake. Participant characteristics (age, sex, stature and mass) were entered into the device prior to measurement. Sixty second sampling epochs were collected at a 30 Hz sample rate and the raw acceleration data from each axis were automatically stored in memory. The Freedson VM3 combination algorithm (Sasaki et al., 2011) was used to estimate physical activity energy expenditure from the vector magnitude counts per minute of the three axis.

While participants were asked to wear the accelerometer at all times (except water related activity; for example washing and swimming), accelerometers were removed periodically for legitimate reasons; for example discomfort during sleep or when it was prohibited during performances. The loss of data associated with removal of the accelerometer influences the estimation of physical activity (Catellier et al., 2005). As recommended previously (Ottevaere et al., 2011), in order to account for missing data during these periods, participants were required to register all non-wear periods in a diary (Ottevaere et al., 2011). This included the time and duration of removal (which was verified with the non-wear accelerometer times) as well as a description of the activities done. Appropriate Metabolic Equivalent (MET) values from the Compendium of Physical Activities (Ainsworth et al., 2011) were assigned to these reported activities, and were subsequently corrected to account for individual

variation (age, sex, stature and mass) (Kozey, Lyden, Staudenmayer, & Freedson, 2010). These corrected METs were used to estimate non-wear energy expenditure (Ainsworth et al., 2011). As with recording dietary intake, participants were specifically instructed to follow typical physical activity patterns during the data collection period.

As well as exercise energy expenditure, both basal metabolic rate (BMR), and the thermic effect of food (TEF) contribute to total daily energy expenditure (Leenders et al., 2001). The Harris-Benedict equation (Harris & Benedict, 1918) validated elsewhere (Roza & Shizgal, 1984) is the most widely used predictive equation (Frankenfield et al., 1998) and was used to estimate BMR. The TEF varies among macronutrients; that of lipids, carbohydrate, and protein equates to 2-3, 6-8, and 25-30% of their intake, respectively (Jequier, 2002). As applied in previous research (Russell & Pennock, 2011), average values were used to calculate the total TEF in the present study (2.5, 7, and 27.5% for lipids, carbohydrate and protein, respectively).

3.2.6 Statistical analysis

All data are presented as mean ± SD unless otherwise stated. Linear regression analysis was conducted on a number of variables from the questionnaires (described in section 3.2.2) thought to predict energy balance over the total 7-day data collection period, and Pearson's product moment correlation coefficients were determined. The physical activity energy expenditure estimated from the accelerometer and from non-wear activity, BMR, and TEF were combined to estimate individual total energy expenditure (TEE). The average TEE (kcal, MJ), TEI (kcal, MJ), energy balance (kcal, MJ), energy availability (kcal·kg FFM^{-1}), and macronutrient contributions (% of TEI, g and $g \cdot kg^{-1}$) were determined for three periods; the total 7-day data collection period, an average day of the week (scheduled dance training), and an average weekend day (no scheduled dance training). The Shapiro-Wilk test of normality was used to establish whether data was normally distributed. This was interpreted in conjunction with quantile-quantile plots to consult the shape of the distribution and determine values of skewness and kurtosis (Field, 2013). Three separate paired samples *t* tests were used to compare

energy intake and energy expenditure during each of the three periods (7-day period, day of the week, and weekend day) in order to assess energy balance. Paired samples *t* tests were also conducted on all variables to compare an average week day and an average weekend day. Where appropriate, Cohen's D effect sizes (*d*) were calculated with the magnitude of effects considered small (0.2), medium (0.5) and large (> 0.8). Statistical software (IBM Statistical Package for Social Sciences (SPSS) v22, IBM, USA) was used and significance accepted at the $p \leq 0.05$ *a priori*.

3.3 Results

3.3.1 Participant demographics

Participant characteristics are presented in Table 4. The Healthier Dance Practice National Survey determined that seven participants were either vegan, vegetarian or actively avoided red meat, six reported to not drink alcohol, two were following weight-reducing diets, and nine reported past and/or current eating problems. Regression analysis determined that these were not significant predictors of energy balance, however past and/or current eating problems was significantly correlated with energy balance ($r = -0.392$, $p = 0.026$). Despite this, participants demonstrated average levels of cognitive restraint (49 ± 20%), uncontrolled eating (44 ± 14%), and emotional eating (47 ± 22%), as evidenced by the TFEQ-R18. Multiple regression analysis (forced entry method) determined that uncontrolled eating ($t_{21} = -2.263$, $p = 0.034$), and emotional eating ($t_{21} = -2.150$, $p = 0.043$) were significant predictors of energy balance. Pearson's product moment correlation coefficients also showed that uncontrolled eating ($r = -0.440$, $p = 0.014$) and emotional eating ($r = -0.445$, $p = 0.013$) were significantly correlated with energy balance. Self-reported menstrual function determined that 14 were eumenorrheic, nine were oligomenorrheic, and two could not be determined due to contraceptive hormone use. Contraceptive use varied; 16 were naturally menstruating, six were using combination pill/vaginal ring/contraceptive patch, two were using progesterone only pills/implant, and one used an oestrogen replacement. Neither menstrual function nor contraceptive use were significant predictors of energy balance. There was difficulty in identifying the menstrual cycle phase in seven

participants (due to irregular cycles or particular contraceptive hormone use) and were therefore undetermined, while nine were in the follicular phase, four in the luteal phase, and five in late luteal and early follicular phases during the 7-day data collection period. Additional analysis to investigate differences in eating and exercise behaviours across menstrual cycle phases could not be performed given the difficulties in attaining this information in this population.

Table 4. Participant characteristics, *n*=25, mean ± SD.

Age (y)	21 ± 2
Body mass (kg)	63.4 ± 6.9
Stature (cm)	167.4 ± 5.9
BMI ($kg \cdot m^{-2}$)	22.6 ± 2.0
Waist : Hip	0.74 ± 0.03
Body fat (%)	28.0 ± 3.4
Fat free mass (kg)	45.5 ± 4.3
Self-reported physical activity ($h \cdot week^{-1}$)	26.3 ± 5.8
Dance training $\geq$ 10 $h \cdot week^{-1}$ (y)	5 ± 3

3.3.2 Energy intake and energy expenditure

Total energy expenditure, and energy and macronutrient intakes are summarised in Table 5. Figure 6 illustrates the daily energy intake and expenditure of each individual across the 7-day period.

Table 5. Daily energy expenditure and energy and macronutrient intakes[1], *n*=25, mean ± SD.

Variable		7-day	Week	Weekend
Energy				
Expenditure	kcal	2784 ± 569	2719 ± 407	2633 ± 574
	MJ	11.6 ± 2.4	11.4 ± 1.7	11.0 ± 2.4
Intake	kcal	2428 ± 458	2297 ± 492*	2756 ± 669*
	MJ	10.2 ± 1.9	9.6 ± 2.1*	11.5 ± 2.8*
Balance	kcal	-356 ± 668	-422 ± 513*	123 ± 1007*
	MJ	-1.5 ± 2.8	-1.8 ± 2.1*	0.5 ± 4.2*
Availability	kcal·kg FFM^{-1}	26 ± 13	24 ± 10*	36 ± 21*
Carbohydrate	g	313 ± 58	304 ± 57	335 ± 97
	$g \cdot kg^{-1}$	5.0 ± 1.0	4.8 ± 0.8	5.4 ± 1.7
	%TEI	52 ± 7	54 ± 7*	49 ± 8*
Protein	g	81 ± 15	79 ± 17	85 ± 22
	$g \cdot kg^{-1}$	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.5
	%TEI	13 ± 2	14 ± 2†	13 ± 3†
Fat	g	92 ± 30	85 ± 33*	110 ± 33*
	$g \cdot kg^{-1}$	1.5 ± 0.4	1.3 ± 0.5*	1.8 ± 0.6*
	%TEI	34 ± 5	32 ± 6*	36 ± 6*
Alcohol	g	9 ± 13	5 ± 14*	20 ± 22*
	$g \cdot kg^{-1}$	0.2 ± 0.2	0.1 ± 0.3*	0.3 ± 0.4*
	%TEI	3 ± 4	2 ± 5*	5 ± 5*

[1]As determined using dietary analysis software (Nutritics Ltd, Swords, Ireland). %TEI, percentage of total energy intake; FFM, fat free mass. *$p < 0.05$, †$p = 0.051$: difference between week and weekend.

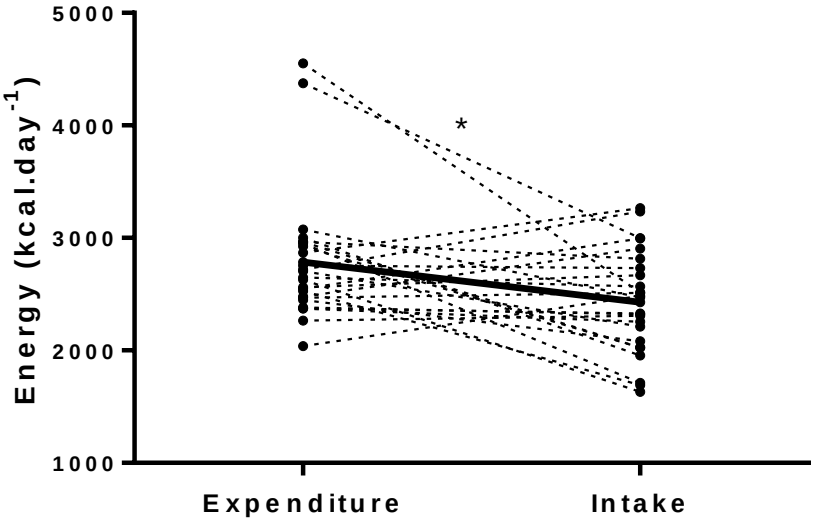

Figure 6. Energy intake and energy expenditure for each individual (*n* = 25) over the 7-day data collection period, and the group mean (dashed line). *denotes group mean significant difference (p < 0.05).

Average energy intake was lower than energy expenditure during the 7-day period (2428 ± 458 kcal or 10.2 ± 1.9 MJ vs 2784 ± 569 kcal or 11.6 ± 2.4 MJ; t_{24} = 2.7, p = 0.014, d = 0.70) equating to an energy deficit of -356 ± 668 kcal·day^{-1} or -1.5 ± 2.8 MJ·day^{-1}. Energy intake was also lower than energy expenditure during the week (t_{24} = 4.1, p < 0.001, d = 0.95) but not during the weekend (t_{24} = -0.6, p = 0.548, d = -0.2). Energy expenditure did not differ when comparing week and weekend days (2719 ± 407 vs 2633 ± 574 kcal; t_{24} = 1.1, p = 0.297, d = 0.18). However daily energy intake (2297 ± 492 vs 2756 ± 669 kcal; t_{24} = -3.4, p = 0.002, d = -0.8) (Figure 7(A)), energy availability (24 ± 10 vs 36 ± 21, kcal·kg FFM^{-1}; t_{24} = -3.3, p = 0.003, d = -0.75), and energy balance (-422 ± 513 vs 123 ± 1007 kcal; t_{24} = -3.2, p = 0.004, d = -0.70) (Figure 7(B)) were lower during the week compared to the weekend, where energy balance in fact became positive.

Two participants demonstrated exceptionally high energy expenditures during the data collection period. Whilst these data greatly deviated from the mean, they were not excluded from the data set given the nature of the cross-sectional study design; intending to observe the reported behaviours of this population. Indeed, a number of studies have previously reported high energy expenditures in dancers (please refer to

Table 1) including values of 4617 ± 1244 kcal·day^{-1} in university level dancers (Hirsch et al., 2003). However, for illustrative purposes, data were reanalysed with these participants removed (n = 23), and it was determined that energy intake remained significantly lower compared to energy expenditure over the 7-day period (t_{22} = 2.1, p = 0.048).

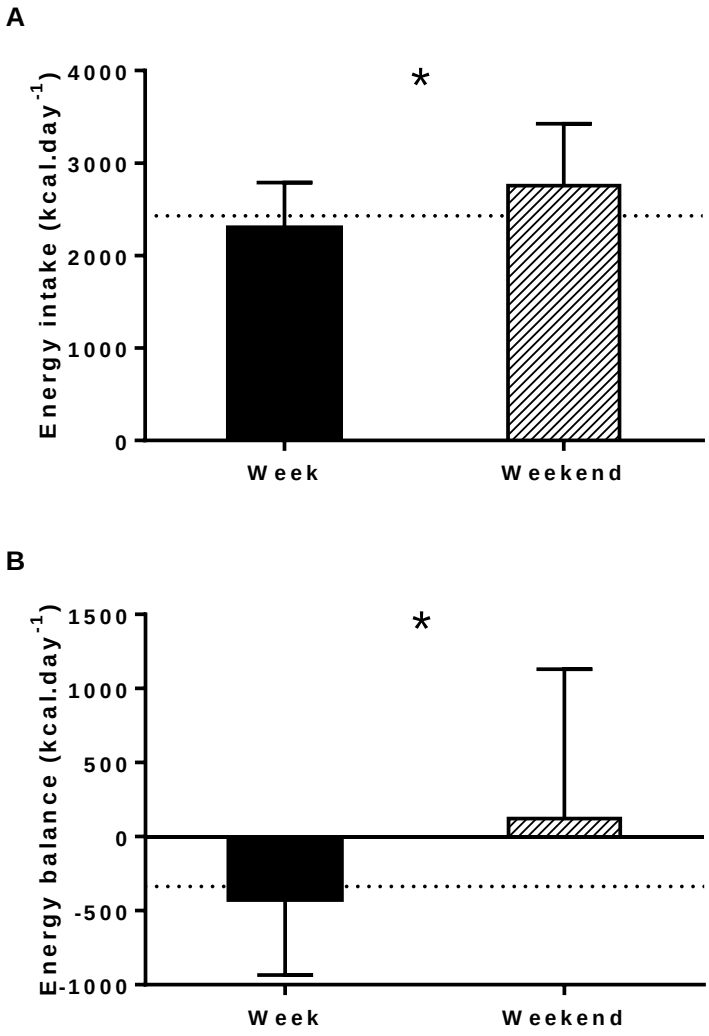

Figure 7. Energy intake (A) and energy balance (B) of participants (*n* = 25) during an average week day, and an average weekend day. The dashed line represents group mean over the total 7-day data collection period. Values presented as mean ± SD. *denotes group mean significant difference between week and weekend (p < 0.05).

Absolute intakes ($g \cdot day^{-1}$ and $g \cdot kg^{-1} \cdot day^{-1}$) of fat ($t_{24}$ = -3.7, p = 0.001, d = -0.76 and t_{24} = -3.7, p = 0.001, d = 0.92, respectively) and alcohol (t_{24} = -3.3, p = 0.003, d = -0.79 and t_{24} = -3.3, p = 0.003, d = -0.58, respectively) were higher at the weekend compared to during the week, while carbohydrate (t_{24} = -1.8, p = 0.083, d = -0.41 and t_{24} = -2.0, p = 0.062, d = -0.46, respectively) and protein (t_{24} = -1.2, p = 0.233, d = -0.32 and t_{24} = -1.4, p = 0.184, d = -0.25, respectively) intakes were not different. Similarly, percentage contributions to TEI (%TEI) of fat and alcohol differed between an average week day and an average weekend day; where fat and alcohol intake was highest at the weekend (t_{24} = -2.5, p = 0.022, d = -0.55 and t_{24} = -2.5, p = 0.020, d = 0.57, respectively), but %TEI of carbohydrate was significantly lower (t_{24} = 3.7, p = 0.001, d = 0.62). There was a strong trend for a lower %TEI derived from protein at the weekend (t_{24} = 2.1, p = 0.051, d = 0.53) as illustrated in Figure 8.

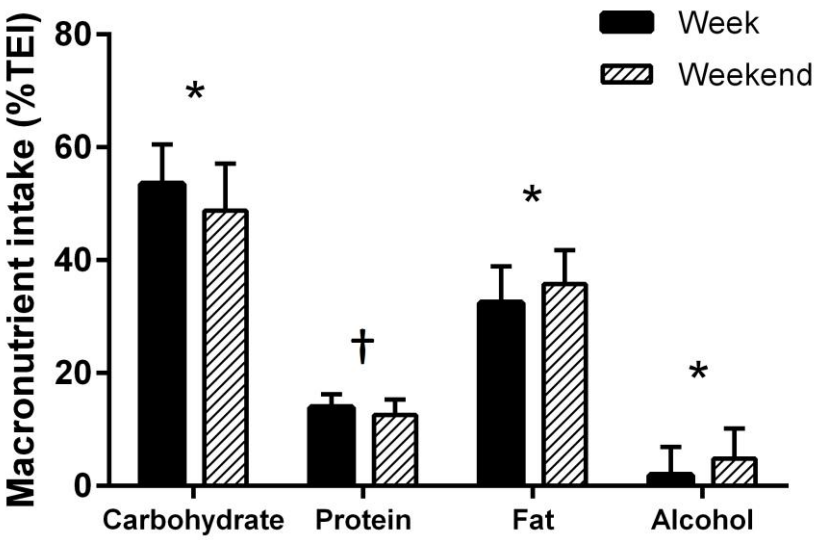

Figure 8. Percentage contributions to total energy intake (%TEI) of carbohydrate, protein fat, and alcohol during an average week day and an average weekend day. Values presented as mean ± SD. *denotes group mean significant difference ($p < 0.05$) and †denotes a trend ($p = 0.051$) towards significant difference between week and weekend.

3.4 Discussion

This study aimed to investigate the energy intake and expenditure of female contemporary dancers during a week of full-time, pre-professional dance training. The present investigation was the first to utilise accelerometry and the combined method of self-report weighed food diaries and dietary recall interview techniques. In agreement with the literature (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002), this study found that on average dancers were in negative energy balance (17 of 25 participants) with a daily deficit of -356 ± 668 kcal (-1.5 ± 2.8 MJ).

The average energy deficit observed in the present study is less than previously reported in some dance populations (recently a deficit of ~2.3 ± 1.4 $MJ \cdot day^{-1}$ in female ballet dancers (Beck, Mitchell, et al., 2015)). However, female dancers (as with other athletic females) are recommended to maintain an energy availability above 30 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ to reduce the risk of disorders associated with energy imbalance (Sousa et al., 2013); the present study demonstrated this to be only 26 ± 13 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$. Chronic energy deficiency and low energy availability in athletes can compromise growth, maturation and health, and lead to detriments in performance and subsequent recovery (Loucks, 2004). The dancers recruited in this study reported to participate in 26.3 ± 5.8 h of exercise activity during an average week (range 17.5 – 42 $h \cdot week^{-1}$). Certainly, in periods which require repeated, high-intensity dance training and performance, the ability for dancers to recovery quickly is vital (Bronner et al., 2016). It is well-documented that both total energy intake and nutritional status influences exercise recovery. Indeed, protein synthesis may be inhibited by energy depletion at the cellular level (Kumar et al., 2009) and the relationship between carbohydrate intake and glycogen resynthesis appears dependent on total energy intake (Tarnopolsky et al., 2001). Moreover, substantial restrictions in energy, protein, and micronutrient intakes may also disturb immune function (Burke, 2010), which might inhibit recovery potential. Accordingly, contemporary dancers could benefit from a greater understanding of their energy requirements and certainly more education regarding appropriate nutritional strategies to support their training demands.

Carbohydrate availability plays an essential role in exercise metabolism and the delay of fatigue, as well as contributing to the replenishment of glycogen stores during recovery. This study observed intakes of 5.0 ± 1.0 $g \cdot kg^{-1} \cdot day^{-1}$ over the 7-day period, which achieved the 5-7 $g \cdot kg^{-1} \cdot day^{-1}$ recommended for athletic populations (Burke, Loucks, & Broad, 2006), however the average 4.8 $g \cdot kg^{-1} \cdot day^{-1}$ during the academic training week falls short of these guidelines. Given that much of contemporary training is typically of low-moderate intensity (Wyon et al., 2011) conducive to beta-oxidation, this is likely sufficient. However, these intakes might be of concern to those in energy deficiency in light of the fact that restricted eating behaviours and inadequate energy intake (predominantly evident in female athletes), can compromise optimal glycogen storage capacity post-exercise (Burke, Kiens, & Ivy, 2004). Therefore, dancers should consider increasing total energy intake in order to maximise glycogen synthesis, particularly during high-intensity training and/or performance periods where physiological demands are higher and when recovery is short. Similarly, adequate protein intake and amino acid availability are necessary for the repair and remodelling of skeletal muscle and connective tissue after exercise (Beelen et al., 2010), which is critical given that dance has been shown to induce muscle damage (Rodrigues-Krause et al., 2014). The average intakes of 1.3 ± 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ in the current investigation meet recommendations of 1.2-1.7 $g \cdot kg^{-1} \cdot day^{-1}$ (Tipton & Wolfe, 2004). However, recent research demonstrates that muscle protein synthesis is down-regulated when in energy deficiency and as a result, those in energy deficit should consume higher protein diets (1.6-2.4 $g \cdot kg^{-1} \cdot day^{-1}$) to restore muscle protein synthesis and attenuate proteolysis and skeletal muscle loss (Pasiakos et al., 2013; Pasiakos, Margolis, & Orr, 2015). This evidence suggests that intakes in this population are likely below optimal, particularly given that 7 of the 25 participants were vegan, vegetarian or actively avoided red meat. Therefore, the protein sources that these participants consumed were likely of predominantly low biological value, for instance in vegetables, legumes, nuts, and grains. This suboptimal protein intake might have implications on the recovery of contemporary dancers.

Lipids play a role in many physiological processes, and oxidation of free fatty acids derived from intramuscular triglycerides provide an energy source for muscle contraction (Watt, Heigenhauser, & Spriet, 2002). However fat intake represented

34 ± 5% of TEI (1.5 ± 0.4 g·kg^{-1}·day^{-1}) and was higher still at the weekend (1.8 ± 0.1 g·kg^{-1}·day^{-1}; representing 36 ± 6% of TEI); above recommended levels of < 30%. Similarly, alcohol intakes were relatively high, contributing 3 ± 4% and 5 ± 5% of TEI across the 7-day period and on an average weekend day, respectively. Evidence suggests that alcohol intake is associated with reduced muscle protein synthesis (Parr et al., 2014), impaired glycogen restoration (Burke et al., 2003), and exacerbated losses in muscle function (Barnes, Mundel, & Stannard, 2010). Certainly, the dancers would benefit from a reduction in alcohol intake, specifically limiting intake to 0.5 g·kg^{-1} in any post-exercise period in order to avoid interference with recovery processes (Barnes, 2014).

Interestingly, the eating behaviours of these dancers are somewhat different between weekdays and the weekend. While this study has demonstrated energy deficits throughout a typical week, the maintenance of similar energy demands (as a result of many of the dancers seeking extra-curricular classes and/or training) and increased energy intake contributed to a positive energy balance during the weekend (123 ± 1007 kcal·day^{-1} or 0.5 ± 4.2 MJ·day^{-1}). Perhaps the dancers perceived that while they were not in training they could indulge in arguably less desirable nutritional behaviours; with higher %TEI from fat and alcohol, lower %TEI from carbohydrate, and trends for lower %TEI from protein. In contrast, during periods of academic dance training, the participants appeared to respond with below optimal energy and macronutrient intakes for their training demands. Though restrictive, uncontrolled, and emotional eating behaviours appeared not to be elevated (49 ± 20%, 44 ± 14%, and 47 ± 22%, respectively), uncontrolled and emotional eating were significantly correlated with, and predictors of energy balance. Moreover, they were somewhat higher than previously reported in adult populations (Anglé et al., 2009; de Lauzon et al., 2004; Keskitalo et al., 2008). For instance, a large scale study demonstrated that a community-based cohort of healthy (21 ± 1 kg·m^{-2}) female young adults (age range 14-27 y, *n* = 163) reported cognitive restraint, uncontrolled eating, and emotional eating levels of 34 ± 20%, 35 ± 19%, and 46 ± 20%, respectively (de Lauzon et al., 2004). In addition, a relatively high proportion of participants in the current study reported past and/or current eating problems (9 of 25 participants), which may be responsible for the erratic eating behaviours; particularly as this was significantly correlated with energy balance

(r = -0.392, p = 0.026). The typical training schedule of a dancer also offers unpredictable and/or limited opportunities for food and drink consumption, likely exacerbating these issues. Indeed, an early investigation suggested that binge eating, particularly at the weekend, might explain why dancers do not experience reductions in weight (Calabrese et al., 1983). Moreover, it is possible that the fluctuations in energy balance and macronutrient contributions observed in this study from day-to-day (week day/weekend) are occurring in the long term (term-time/off-season). Certainly, an inherent limitation of cross-sectional study design is that it offers information pertaining to a limited period. The behaviours observed in the current study would be expected to differ if measured at different times of year (for example at the beginning of the academic year when students are likely less fatigued as opposed to the end), and if measured longitudinally. These results should be interpreted with this in mind (please refer to section 7.3). This may explain why the dancers' body composition reported in the present investigation were healthy, despite an average negative energy balance indicative of weight loss. Certainly, BMI and percentage body fat of the participants were higher than typically reported in ballet populations; for instance, 18.9 ± 1.0 kg·m^2 and 17.4 ± 3.4%, respectively in female ballet dancers (van Marken Lichtenbelt et al., 1995). However, given the differences in physiological demands as well as discrete skills between these dance genres (not least the gender roles in ballet requiring females to be lifted more frequently) (Wyon et al., 2011), this is perhaps unsurprising. Indeed, a study which recruited a large cohort of trained dancers demonstrated that female ballet dancers had lower body mass (50.4 ± 4.4 vs 55.7 ± 6.3 kg), BMI (18.7 ± 1.3 vs 20.8 ± 1.8 kg·m^{-2}), percentage body fat (%BF) (17.5 ± 2.5 vs 21.2 ± 3.8%) and were less muscular (3.4 ± 1.1 vs 4.1 ± 1.0 Mesomorphy Rating Scale) than contemporary counterparts (Liiv et al., 2013). Similarly, a more recent study (Bronner et al., 2014) demonstrated differences in BMI of professional ballet and modern dancers (20.8 ± 2.2 kg·m^{-2} vs 22.6 ± 2.0 kg·m^{-2}, respectively). Collectively, these data suggest that the dancers are unable to effectively regulate their energy and macronutrient intakes to accommodate their energy expenditure which is essential for maintaining the demands of training, performance, recovery, and for physiological adaptation.

Accurately quantifying energy intake and energy expenditure is limited by indirect measurement techniques typically relied upon in research studies. In the present

study, a 7-day period was chosen as it is thought to best represent the variety of dietary and physical activity practices and are associated with the most valid nutritional information (Bingham, 1987). However, in order to minimise the measurement errors associated with participant compliance and motivation, this study combined self-reported weighed food diaries with 24-hour recall interviews. This method has been found to have good agreement with the gold standard observed food intake technique (Briggs, Rumbold, et al., 2015; Rumbold et al., 2011). Moreover, accelerometry has been shown to be strongly correlated with indirect calorimetry (Jarrett, Fitzgerald, & Routen, 2015; McMinn et al., 2013) however, some show an underestimation of physical activity levels using the devices (Ainsworth et al., 2000; Macfarlane, Lee, Ho, Chan, & Chan, 2006; Sirard, Melanson, Li, & Freedson, 2000). Notwithstanding, the loss of data associated with removal of the accelerometer evidently influences the estimation of physical activity (Catellier et al., 2005). Though efforts were made to account for missing data using non-wear activity logs, the accuracy of these is nevertheless limited by the possibility that, 1) not all activities were reported and accounted for, and 2) the information provided was lacking in detail. It is also important to note that demanding exercise and energy expenditure may alter BMR; particularly during energy deficit. Future research should consider more accurate estimation of BMR to determine its contribution to TEE. Similarly, energy balance may differ across the menstrual cycle, for instance energy intake and expenditure appear to be elevated during the luteal phase (Davidsen, Vistisen, & Astrup, 2007). In the present investigation, the menstrual cycle phase of participants during the study differed and in some cases was not identifiable (due to irregular cycles or particular contraceptive hormone use), and therefore the findings should be interpreted with this in mind. Finally, it would have been valuable to determine the energy and macronutrient intakes consumed by the dancers surrounding their training, specifically in the minutes and hours post-exercise. However, given that the training schedule of the dancers in the present investigation often required them to attend training/performance sessions in quick succession with little (and in some cases no) rest, it was difficult to identify pre and post-exercise nutritional intakes. Despite the aforementioned limitations, the present study offers a high degree of ecological validity in its free-living experimental design, which may not have been possible with other experimental techniques.

3.5 Perspectives

This chapter addressed the first aim of the thesis: '*to determine the typical training and eating behaviours of pre-professional female dancers*'. The results from this study resulted in the rejection of the null hypothesis, concluding that there was a significant difference between energy intake and energy expenditure of pre-professional female dancers. This study demonstrated for the first time that as with many athletes in aesthetic or weight dependent sports, and as observed in other dance populations, female contemporary dancers are at risk of energy deficiency, particularly during periods of scheduled dance training. As a result, this population could be susceptible to numerous health, performance, and recovery impairments associated with energy imbalance. In addition, the suboptimal macronutrient intakes observed in this study suggest a lack of knowledge regarding appropriate nutrition for sport and exercise activity. It is evident that dance populations would benefit from further research in order to develop current understanding of dance specific nutrition. Moreover, this study has demonstrated that the training schedule of pre-professional female contemporary dancers is demanding, and consequently, it is conceivable that they are at risk of muscle damage and its associated negative symptoms. This question will be examined in the following experimental chapter 4. Indeed, this study suggests that it may be prudent to investigate the use of nutritional strategies, which may provide practical solutions to improve energy balance and perhaps contribute to enhancing recovery. This would be of particular value when dancers are expected to participate in multiple daily training/performance sessions and on several consecutive days with limited recovery. Potential nutritional interventions will be explored in chapters 5 and 6.

4 Exercise-induced muscle damage following dance-specific and repeated-sprint exercise in female dancers

Publication arising from this chapter:

Brown, M. A., Howatson, G., Keane K., & Stevenson, E. J. (2016). Exercise induced muscle damage following dance and sprint exercise in females. *J Sports MedPhys Fitness, 56*(11), 1376-1383.

4.1 Introduction

Exercise-induced muscle damage (EIMD) is commonly experienced in sport and exercise, and has important implications on the quality of subsequent training and performance. There are various symptoms associated with EIMD, particularly following eccentric-biased activity (Clarkson & Hubal, 2002), including performance decrements, and increases in muscle soreness, inflammation, and systemic appearance of intramuscular proteins (Howatson & van Someren, 2008). These signs and symptoms, which persist for several days (Armstrong, 1984), are thought to be attributed to an initial mechanical disruption during the exercise insult and a secondary inflammatory response (Howatson & van Someren, 2008); the magnitude of which is dependent on the mode, intensity and duration of exercise (Proske & Morgan, 2001) as well as an individual's training status (Tee et al., 2007). A popular method to induce muscle damage is using single-limb isokinetic contractions, conducted in controlled laboratory conditions (Howatson et al., 2007). This lacks sporting specificity and might not be wholly applied to sport and exercise performance. In addition, though the damage responses have been well established in male populations (Goodall & Howatson, 2008; Howatson, Hoad, et al., 2012; Howatson & Milak, 2009; Kanda et al., 2013), there is a paucity of literature investigating EIMD in females. Various factors are considered to influence the EIMD response in females that include oral contraceptive use and the potential protective effect of oestrogen (Tiidus, 2000). Additionally, the secondary inflammatory response might be sex dependent, with reports of sex differences in leukocyte and cytokine infiltration into skeletal muscle post-exercise (Peake, Nosaka, & Suzuki, 2005; Tiidus, 2003). It therefore makes the expectation tenable that the damage response in females could be somewhat different to males; and so it is important to ascertain the consequences of conducting strenuous and potentially damaging exercise in females.

Although there are divergences between genres, dance is characterised as an intermittent and moderate-high intensity form of exercise (Wyon, Head, Sharp, & Redding, 2002) with a high frequency of eccentric contractions (Paschalis et al., 2012; Westblad et al., 1995); likely exposing dancers to EIMD. According to the Sport and Recreation Alliance (2014) there are 5.5 million adults recreationally

participating in dance-type activity in the UK alone. Despite the popularity of dance (both recreationally and professionally), only one study (Rodrigues-Krause et al., 2014) has examined the damaging consequences precipitated from a dance rehearsal and performance. Although this investigation reported an increase in creatine kinase following dance, measures of muscle soreness and muscle function were not investigated and therefore the potential effects on subsequent performance are less clear. The scarcity of data investigating the damaging effects of dance activity is surprising given the demands of training and performance previously reported in dance populations (Twitchett et al., 2010; Wyon, 2010). While there are some sport-specific paradigms that have been used to elicit EIMD (for instance a simulated rugby match (Twist & Sykes, 2011), a marathon race (Howatson et al., 2010) and a sport-specific repeated-sprint test (Howatson & Milak, 2009)), a suitable and replicable model that is specific to dance has not yet been explored and warrants investigation. Certainly, individuals participating in dance-type exercise would benefit from an increased understanding of how exposure to activity-specific stimuli might lead to symptoms associated with muscle damage.

Consequently, the aims of this study were to firstly determine if a dance-specific protocol adapted from Redding et al. (2009) induces a muscle damage response in female dancers; and secondly to compare the magnitude of damage to that elicited by a more conventionally used, sport-specific repeated-sprint activity that has been shown to cause EIMD. These exercise modes differ in a number of respects, for instance dance is characterised by complex movement sequences often pre-choreographed, while the nature of repeated-sprint sports means that movements are much more unpredictable. However, both activities involve accelerations and decelerations and changes of direction that have been previously shown to elicit muscle damage (Howatson & Milak, 2009; Keane, Salicki, et al., 2015; Rodrigues-Krause et al., 2014). Therefore, it was hypothesised that a dance-specific exercise bout would cause muscle damage and the magnitude of this response would be comparable to a traditional bout of damaging exercise. As such this chapter sought to address the second aim of the thesis: *'to examine the exercise-induced muscle damage response to both dance-specific and sport-specific exercise in female dancers'*.

4.2 Materials and methods

4.2.1 Participants

4.2.1.1 Recruitment

Female recreational dancers (defined as those who took part in regular dance training for recreation; outside of academic or work commitments) from a university dance team and aged between 18-21 years were approached. This dance team held auditions each year, and while their dance background differed, all recruited members had been dancing recreationally for a number of years and were therefore not novice dancers. The dance team trained in a number of dance styles, including largely in modern and contemporary dance. Recruitment was initiated through email, social networking, and through verbal communication to the population described. Interested individuals were provided with detailed participant information sheets outlining aims, objectives and methodology as well as a list of contraindications to ascertain any medical or physical conditions that would exclude them from participation. Exclusion criteria were; epilepsy, bronchitis, severe asthma, cardiac complaints, bacterial or viral infection in the 2 weeks preceding, injury or recovering from an injury sustained in the preceding 4 weeks, pregnancy, food allergy (as discussed with the investigator), or anything that may prevent them from successfully completing the study that was described. Contraceptive use (regardless of form) was not an exclusion criterion. In addition, associated risks and benefits were discussed prior to gaining written informed consent (Appendix A).

4.2.1.2 Sample size

Maximal isometric voluntary contraction (MVC) peak force or torque is suggested to be the best measure of muscle damage resulting from eccentric contraction, and provides the primary means for determining muscle function (Warren et al., 1999). The sample size was determined by completing a power analysis (power = 0.8, $\alpha = 0.05$) based on isometric strength data from Howatson and Milak (2009). This determined that a sample size of 8 in each group would provide

statistical power above 80%, with an alpha level of 0.05. In order to account for dropouts, the aim was to recruit a sample of 10 per group.

4.2.1.3 Participant characteristics

Twenty-nine healthy female recreational dancers (mean ± SD age 19 ± 1 y; stature 164.4 ± 3.9 cm; mass 58.8 ± 5.6 kg; and BMI 21.8 ± 2.0 $kg \cdot m^{-2}$, respectively) from a university dance team were recruited and gave written informed consent. Self-reported physical activity levels were determined by the number of hours of dance training and total exercise each week, and training status was determined by the number of years participating in regular dance training (please refer to Table 6). All participants typically attended dance rehearsals twice per week (5.4 ± 2.9 $h \cdot week^{-1}$). A 3-day food diary and activity log completed prior to testing determined that there were no differences in physical activity levels or energy and macronutrient intakes between participants (all characteristics displayed in Table 6). Participants were asked to replicate their reported diets as closely as possible throughout the testing period. The study was conducted according to the guidelines of the Declaration of Helsinki and all experimental procedures were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria (RE-HLS-13-131119-528b6f3d47f50).

Table 6. Participant characteristics, mean ± SD.

Variable		Total (n = 29)	DP (n = 15)	SP (n = 14)	p value[1]
Characteristics					
Age (y)		19 ± 1	20 ± 1	19 ± 1	0.176
Body mass (kg)		58.8 ± 5.6	57.4 ± 6.1	60.0 ± 4.9	0.183
Stature (cm)		164.4 ± 3.9	162.4 ± 3.6	166.5 ± 3.1	0.003
BMI ($kg \cdot m^{-2}$)		21.8 ± 2.0	21.8 ± 2.1	21.8 ± 2.0	0.990
Dance training (y)		13 ± 1	14 ± 3	11 ± 5	0.055
Dance training ($h \cdot week^{-1}$)		5.4 ± 2.9	5.5 ± 2.7	5.4 ± 3.1	0.946
Total exercise ($h \cdot week^{-1}$)		8.3 ± 3.7	8.2 ± 2.3	8.4 ± 4.7	0.873
Average daily intakes[2]					
Energy	kcal	1550 ± 456	1486 ± 334	1619 ± 564	0.456
	MJ	6.5 ± 1.9	6.3 ± 1.4	6.8 ± 2.3	0.456
Carbohydrate	$g \cdot kg^{-1}$	3.4 ± 1.1	3.6 ± 0.9	3.3 ± 1.4	0.609
	%TEI	52 ± 7	55 ± 5	50 ± 8	0.087
Protein	$g \cdot kg^{-1}$	1.2 ± 0.5	1.1 ± 0.4	1.2 ± 0.6	0.749
	%TEI	18 ± 5	17 ± 5	18 ± 6	0.904
Fat	$g \cdot kg^{-1}$	0.9 ± 0.4	0.9 ± 0.4	1.0 ± 0.4	0.573
	%TEI	32 ± 6	30 ± 6	33 ± 7	0.333

[1]Dance-specific protocol (DP) vs repeated-sprint protocol (SP), compared by independent samples *t* test. [2]As determined using dietary analysis software (Nutritics Ltd, Swords, Ireland) from a 3-day food diary prior to data collection. %TEI, percentage of total energy intake.

4.2.1.4 Dietary and exercise restrictions

For 48 h prior to, and for each of the testing days, participants were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements (including vitamin and mineral tablets), and any anti-inflammatory drugs or alternative treatments (including massage and cold water immersion). While participants were not provided with an exhaustive list of supplements and treatments to avoid, they were instructed to contact the investigator who would clarify whether items were authorised, should they have any queries. These restrictions were employed to limit the influence of diet and physical activity on the dependent variables and ensured that observed effects were likely to be in response to the exercise implemented within the study.

4.2.2 Pre-testing procedures

Upon receiving written informed consent, participants were required to complete a menstrual cycle questionnaire (Appendix C). This identified the contraceptive use of participants; 19 were using an oral combination pill (all monophasic), five were using a progesterone only pill/implant/injection, and five were menstruating normally. This also determined menstrual cycle phase; all data collection took place during the early to mid-luteal phase, or where applicable in the 14 days before a withdrawal bleed. This was to avoid the peak in ovarian oestrogen observed 7-10 days into the pill cycle following a pill-free interval; common (~20% of cycles) with cyclical regimes (Legro et al., 2008). Participants were initially required to attend the laboratory for familiarisation with the procedures and the exercise protocol was described. Familiarisation with the dependent variables was important to minimise systematic error associated with the learning and practice effect (Hopkins, 2000; Hopkins, Schabort, & Hawley, 2001). Finally, participants were required to complete three maximal voluntary isometric contractions (MVC) of the knee extensors (described in detail in section 4.2.5.3.3). The peak MVC was used to create two equal and homogenous strata of 'strong' and 'less strong' participants. Stratified randomisation was then used to assign participants to groups from each of these strata in order to ensure they were matched and counterbalanced for muscle function. Following these pre-testing procedures (~1-2 weeks prior to data collection), participants were then required to attend the laboratory on four further occasions and were tested at the same time on subsequent days (± 1 h) to account for diurnal variation.

4.2.3 Experimental protocol

This study adopted an independent groups design and used stratified randomisation (described in section 4.2.2) to assign participants to one of two groups; either a dance-specific exercise bout (DP; n = 15) or a sport-specific repeated-sprint protocol (SP; n = 14). Following written informed consent and pre-testing procedures (described in section 4.2.2), participants attended the laboratory for a further four consecutive days. Participants were fasted for ≥2 h prior to each laboratory visit,

except for water (which was consumed *ad libitum*). The first visit determined participant characteristics; stature was measured to the nearest 1 mm (stretch stature technique, Model 220, Seca Ltd, Birmingham, UK) and body mass to the nearest 0.1 kg (Model 876, Seca Ltd, Birmingham, UK). Participants then completed baseline measurements involving a battery of commonly used muscle damage indices. This was followed by a bout of exercise designed to induce muscle damage and after a 2 min rest, measurement of dependent variables were repeated (0 h post EIMD). For the three subsequent days following muscle-damaging exercise, participants returned to the laboratory to repeat baseline measures and these were carried out in the same order (24, 48 and 72 h post EIMD). Please refer to Figure 9 below for an illustration of the study design and the following sections for details regarding all data collection procedures. Protocols and measurement of dependent variables were completed in an indoor sporting facility and environmental conditions were controlled (temperature, 17.6 ± 0.2^0C; pressure, 1011.5 ± 9.9 hpa).

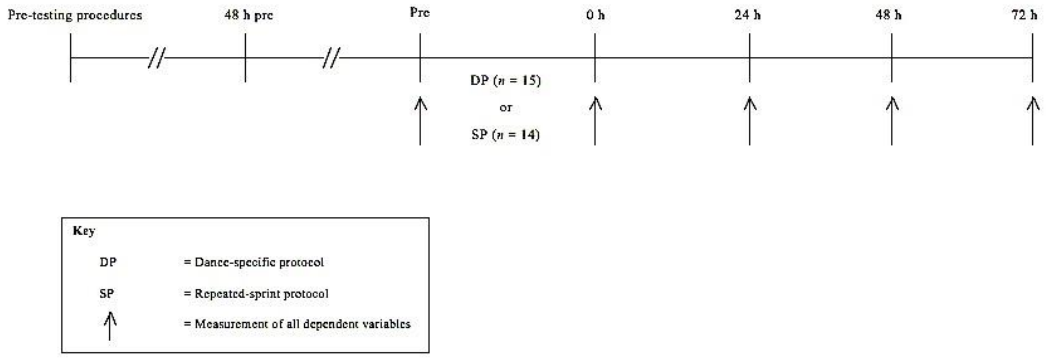

Figure 9. Schematic of testing protocol.

4.2.4 Exercise protocols

4.2.4.1 Standardised warm-up

Prior to baseline measurement of muscle function and prior to exercise protocols, participants completed a standardised warm up. This comprised of 400 m of jogging at a self-selected pace, a series of sprint drills (including high knees, heel flicks, and

walking lunges) and three practice sprints (Glaister, Howatson, Abraham, et al., 2008; Glaister et al., 2007; Glaister, Howatson, Pattison, & McInnes, 2008). Participants were also given 5 minutes to perform any personal stretches and prepare themselves for measurement of muscle function and the assigned protocol. Each participant's individual warm up on the initial day was noted so this could be replicated throughout testing. Standardised instructions and strong verbal encouragement from the investigator to encourage maximal effort were provided throughout each muscle-damaging protocol. Participants completed either the DP (n = 15) or the SP (n = 14) to induce muscle damage.

4.2.4.2 Dance-specific protocol (DP)

The *dance performance fitness test* was developed to assess and monitor dancers' cardiovascular fitness (Redding et al., 2009) and consists of a great deal of eccentric muscle actions. Hence it was thought to provide a potential activity-specific model to induce muscle damage in dancers. This test described previously (Redding et al., 2009) involves the repetition of a dance phrase representative of contemporary dance at a tempo of 106 $b \cdot min^{-1}$, with each phrase separated by a 2 min rest period. The test protocol consisted of jumps in first and second position, rolls to the floor, weight transference from feet to hands and back to feet, circular springs with an arm pattern, and a parallel jump forward in space using an arm swing. For the purpose of the study, the originally described test was repeated twice, to be more representative of the duration of a dance and therefore of the muscle damage that might be experienced following such exercise. Specifically, contemporary dance has been reported to typically last 24 ± 7 min (Wyon et al., 2011) and is predominantly an intermittent type of exercise with rest periods (Wyon et al., 2002). The adapted protocol took 30 min to complete; 10 x 1 min dance phrase separated by 2 min rests.

Participants assigned to this dance-specific protocol (DP) were taught the dance sequence via video recordings as well as by the investigator. However, they were asked to 'mark' the movements rather than perform with maximal effort so as to ensure that there was no repeated bout effect associated with this exercise before the testing began. Participants were required to complete the DP to a musical

accompaniment at a tempo of 106 b·min^{-1} and to maintain this intensity throughout the test.

4.2.4.3 Repeated-sprint protocol (SP)

Two sets of light timing gates (Brower telemetric timers, Brower timing systems, Draper, USA) were set at each end of a 30 m section of a 50 m running track. A further 10 m deceleration zone was also marked on either side of the end of the 30 m sprint section. Participants stood 30 cm from the start line (marked with tape) to avoid premature triggering of the timing system. The repeated-sprint protocol (SP), comprised 15 x 30 m sprints with a rapid 10 m deceleration phase, each separated by 60 s rest (Howatson & Milak, 2009). Participants were instructed to sprint maximally between the light gates and to stop within the 10 m deceleration zone. The 60 s rest period was initiated when the repetition was completed (i.e., the participant had come to a complete halt). This damage model has been demonstrated to induce muscle damage previously, and is thought to represent the damage that might be experienced following repeated-sprint field sports such as soccer, rugby and field hockey (Howatson & Milak, 2009; Keane, Salicki, et al., 2015). The 15 x 30 m sprint times of those completing the SP were also recorded to determine total sprint time, mean sprint time, and rate of fatigue to ensure that exercise intensity was maintained and not different between groups. Fatigue index was calculated using the following formula (Fitzsimons, Dawson, Ward, & Wilkinson, 1993):

Fatigue index (%) = (100 x [total sprint time/ideal sprint time]) - 100, in which total sprint time = sum of sprint times from all sprints, and ideal sprint time = the number of sprints x fastest sprint time.

4.2.5 Dependent variables

The following dependent variables were measured pre, immediately post (0 h), and 24, 48, and 72 h post muscle-damaging exercise.

4.2.5.1 Active muscle soreness (DOMS)

Subjective delayed onset of muscle soreness (DOMS) was measured using a 200 mm visual analogue scale (VAS) with 'no soreness' and 'unbearably sore' anchored at each end of the scale (Appendix E). On each occasion, participants were required to complete a 90^0 squat with hands on their hips, and upon standing to indicate on the line the level of perceived active lower limb soreness felt. The VAS has demonstrated excellent reliability (Bijur et al., 2001) and distinct thresholds for clinically meaningful changes in acute pain intensity (Gallagher, Liebman, & Bijur, 2001; Todd, Funk, Funk, & Bonacci, 1996). A number of previous research investigations have demonstrated that measuring DOMS with a VAS is sensitive to changes following muscle-damaging exercise (Bell et al., 2015; Cockburn et al., 2013; Howatson et al., 2010).

4.2.5.2 Limb girth

Limb girth was measured as an indirect marker of inflammatory swelling and oedema (Smith, 1991; van Someren, Edwards, & Howatson, 2005). An anthropometric tape measure (Bodycare Products, Warwickshire, United Kingdom) was used to determine lower limb girths. Girths at the calf (measured at its largest girth at baseline) and mid thigh (located as midway between the inguinal fold and the superior border of the patella) of the right leg were recorded. These locations on the skin were marked with permanent marker on the initial day of testing to ensure consistency in measurement on subsequent days. Each girth was measured twice at each time point and if a difference of ± 5% was observed then a third measure was taken. The average of the two closest measurements was used for statistical analysis. Calf and mid thigh girth intra-examiner %CVs were < 1%.

4.2.5.3 Muscle function

4.2.5.3.1 Countermovement jump height (CMJ)

Decreases in vertical jump height have been reported following EIMD (Byrne & Eston, 2002a; Byrne, Twist, & Eston, 2004). Participants completed three countermovement jumps (CMJ) using a light timing system (Optojump, Microgate,

Bolzano, Italy). The Optojump system demonstrates excellent test-retest reliability and a strong concurrent validity (Glatthorn et al., 2011). Participants were asked to squat down (bending at the knee, hip and ankle while keeping their heels on the floor and their back straight) with their feet shoulder width apart and to jump vertically and maximally, keeping their hands on their hips throughout. Participants were asked to keep their legs straight while jumping; only bending once the feet contacted the ground. Each effort was separated by 60 s of rest, and the peak CMJ was used for analysis. Intra-trial and inter-trial %CV was established from reliability testing at < 4% and < 3% respectively.

4.2.5.3.2 Reactive strength index (RSI)

Reactive strength index (RSI) is an appropriate measure of muscle function in sports involving jumps as it illustrates the ability to utilise the stretch shortening cycle (Young et al., 1999) and is a highly reliable method of assessing explosive strength (Ebben & Petushek, 2010). RSI has been used to measure performance in a number of studies (Cockburn, Stevenson, Hayes, Robson-Ansley, & Howatson, 2010; Ebben & Petushek, 2010; Kums, Ereline, Gapeyeva, & Paasuke, 2005). Participants completed three drop jumps from a height of 30 cm separated by 60 s of recovery using a light timing system (Optojump, Microgate, Bolzano, Italy). Participants were instructed to have feet shoulder width apart with their hands on hips throughout. They were asked to drop from the box and upon landing to perform a two-footed jump maximally with minimum contact time. Legs were kept straight while jumping; only bending once the feet contacted the ground. This method has been described previously (Young et al., 1999). RSI was calculated from the jump height (cm) ÷ contact time (s) of each drop jump. The peak RSI from three drop jumps was used for statistical analysis. Intra-trial and inter-trial %CV was established from reliability testing at < 12% and < 6% respectively.

4.2.5.3.3 Maximal voluntary isometric contraction (MVC)

Maximal voluntary isometric force has been used extensively to assess muscle function (Clarkson et al., 1992; Cleak & Eston, 1992b) and has generally shown high reliability (Wilson & Murphy, 1996). Muscle which has been eccentrically exercised is typically unable to generate high levels of force and power (Byrne &

Eston, 2002b) and consequently the detriments and recovery of maximal voluntary isometric contraction (MVC) is frequently measured in studies investigating EIMD (Bell et al., 2015; Howatson & Milak, 2009). MVC of the right knee extensors was measured using a strain gauge (MIE Digital Myometer, MIE Medical Research Ltd, Leeds, UK). While in a seated position, the strain gauge load cell was wrapped immediately above the malleoli (a layer of padding was in place to avoid participant discomfort) and attached securely to a plinth on a purpose-built chair at the same height. The knee joint angle was standardised at 90^0 of flexion using a goniometer and confirmed before each contraction in order to minimise any variations on muscle length and the subsequent torque production (Warren et al., 1999). Participants were sat in an upright seated position and were instructed to keep their left leg still and to remain in this position throughout each effort to exclude contribution from the hip musculature during the contraction. Participants received a verbal countdown of 3 s before extending their knee 'as fast and as hard as possible' (Sahaly, Vandewalle, Driss, & Monod, 2001). Participants were asked to complete three MVCs lasting 3 s, interspersed with 30 s of rest. The peak force (Newtons, N) of the three MVCs was used for analysis. Intra-trial and inter-trial %CV was established from reliability testing at < 4% and 2% respectively.

4.2.5.3.4 Thirty metre sprint time

Participants completed a single maximal effort 30 m sprint at each time point, and sprint time was recorded. The sprint was initiated from a line 30 cm behind the start line to prevent false triggering of the timing gates (Brower telemetric timers, Brower timing systems, Draper, USA). Both intra-trial and inter-trial %CV was established from reliability testing at < 2%.

4.2.5.4 Blood sampling and analysis

Blood samples were collected by means of venepuncture from the antecubital fossa area by the principal investigator or another trained phlebotomist. Participants lay supine for approximately 5 min prior to each blood sample to account for postural changes in plasma volume. Samples were collected into 10 mL ethylenediaminetetraacetic acid (EDTA) tubes (Vacutainer BD UK Ltd, Oxford,

UK). Plasma samples were immediately centrifuged at 3000 relative centrifugal force (RCF) for 15 min at 4^0C (Allegra X-22 Centrifuge, Beckman Coulter, Bucks, UK). Plasma supernatant was then extracted and stored in aliquots at -80°C for later analysis. Twenty-four participants consented to blood collection; n = 12 in both groups. Due to sampling error, where data for a single time point were missing (2 points were missing out of a total of 120 (< 2%)), the group mean was used to complete the data set.

4.2.5.4.1 Total creatine kinase analysis

Plasma total creatine kinase (CK) concentrations were determined spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, Burgess Hill, UK). This used the 'reverse reaction' and activation by acetylcysteine NAC method which is in line with the recommendations of the German Society for Clinical Chemistry and the International Federation of Clinical Chemistry. The measurement range for this method was 7-2000 $IU \cdot L^{-1}$. While expected values vary, it is thought that the normal reference range for healthy adult females is 20-180 $IU \cdot L^{-1}$. The inter-assay and intra-assay %CV were both < 2%.

4.2.6 Statistical analysis

Unless otherwise stated, results are presented as means ± standard deviation (SD). For the purpose of data analysis, all dependent variables except for DOMS and CK are expressed as a percentage change relative to pre muscle damage values to account for inter-individual variability. Statistical software (IBM SPSS V21, IBM, Armonk, USA) was used for inferential analysis and statistical significance was accepted at the $p \leq 0.05$ level *a priori*. The Shapiro-Wilk test of normality was used to establish whether data was normally distributed. This was interpreted in conjunction with quantile-quantile plots to consult the shape of the distribution and determine values of skewness and kurtosis (Field, 2013). Mauchly's test assessed the sphericity of the data and, where appropriate, violations were corrected using the Greenhouse–Geisser correction. Two-way group (2; DP vs SP) x time (5; pre, and 0, 24, 48 and 72 h post EIMD) repeated measures analysis of variance (ANOVA) were performed for each dependent variable to determine the muscle damage response

following both the DP and SP and for comparison of the response between the exercise protocols. Significant main effects were analysed using the Least Significant Difference test (LSD) for adjustment for multiple comparisons. Where appropriate, Cohen's D effect sizes (*d*) were calculated with the magnitude of effects considered small (0.2), medium (0.5) and large (> 0.8).

4.3 Results

All sampling distributions were considered normally distributed and there were no group differences in the absolute pre-exercise values of all dependent variables (independent samples *t* test, all $p > 0.05$). For illustrative purposes, absolute values for all dependent variables are presented in Table 7.

4.3.1 Muscle soreness

There was a main effect of time for DOMS ($F_{2.9, 77.6} = 32.3$, $p < 0.001$). Pre-exercise DOMS was 6.0 ± 11.1 vs 16.6 ± 36.0 mm in the DP and SP groups, respectively ($p = 0.286$) and increased immediately post-exercise, peaking at 24 h post-exercise (90.1 ± 35.2 vs 88.0 ± 47.4 mm in the DP and SP groups, respectively). DOMS remained elevated throughout recovery in the DP group, but returned to near baseline levels by 72 h in the SP group (Figure 10). There were no group (grand mean 50.0 ± 5.5 mm, $F_{1, 27} = 0.1$, $p = 0.966$) or interaction effects ($F_{2.9, 77.6} = 2.4$, $p = 0.077$).

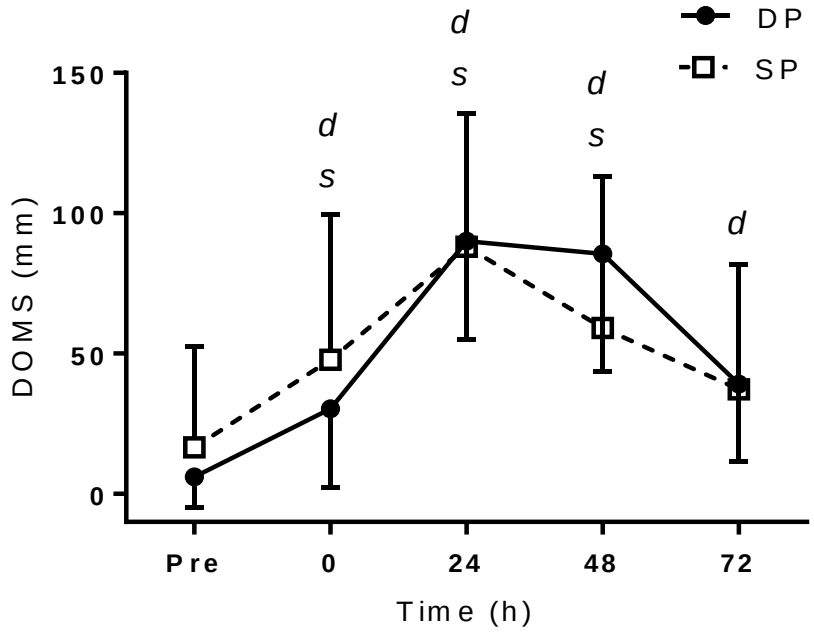

Figure 10. Muscle soreness (DOMS) in response to muscle-damaging exercise in the DP (*n* = 15) and SP (*n* = 14) groups. Values presented as mean ± SD. [d]denotes significantly different from pre-exercise in the DP group. [s]denotes significantly different from pre-exercise in the SP group. Significance at p < 0.05.

4.3.2 Limb girth

Pre-exercise thigh girth was 48.5 ± 3.1 vs 48.5 ± 2.3 cm in the DP and SP groups, respectively (p = 0.980) and pre-exercise calf girth was 36.0 ± 2.1 vs 35.6 ± 1.7 cm in the DP and SP groups, respectively (p = 0.589). Both thigh and calf girths changed over time ($F_{4, 108}$ = 7.4, p < 0.001 and $F_{4, 108}$ = 4.0, p = 0.005, for thigh and calf girths, respectively). Thigh girth increased immediately post EIMD (0 h) and both thigh and calf girth increased 24 h post EIMD compared to pre-exercise in the DP group, but not following SP. There were no differences between groups for thigh and calf girths (grand mean 48.7 ± 0.5 cm, $F_{1, 27}$ = 1.1, p = 0.315; and grand mean 35.9 ± 0.4 cm; $F_{1, 27}$ = 0.1, p = 0.958, respectively) and no interaction effect for calf girth ($F_{4, 108}$ = 2.0, p = 0.097). There was a group x time interaction for thigh girth

($F_{4, 108}$ = 3.4, p = 0.011) where the increase at 0 h in the DP group was greater compared to the SP group (p = 0.014, d = 1.07).

4.3.3 Muscle function

Independent samples t tests determined that there were no significant group differences between absolute pre-exercise values of measures of muscle function (p = 0.687; p = 0.603; p = 0.665; and p = 0.431 for CMJ, RSI, MVC and 30 m sprint time, respectively). All measures of muscle function were reduced post-exercise ($F_{2.0, 53.7}$ = 14.4, p < 0.001; $F_{2.2, 59.4}$ = 3.9, p = 0.022; $F_{2.8, 75.4}$ = 10.0, p < 0.001; and $F_{4, 108}$ = 6.8, p < 0.001 for CMJ, RSI, MVC and 30 m sprint time, respectively) and progressively recovered throughout recovery (Figure 11). While recovery of these measures appeared to accelerate following DP, there were no group effects (grand mean 25.1 ± 0.8 cm, $F_{1, 27}$ = 4.1, p = 0.052; grand mean 64.9 ± 4.2 cm·s^{-1}, $F_{1, 27}$ = 0.1, p = 0.704; grand mean 358.2 ± 11.7 N, $F_{1, 27}$ = 0.6, p = 0.454; and grand mean 5.4 ± 0.1 s, $F_{1, 27}$ = 2.5, p = 0.126 for CMJ, RSI, MVC and 30 m sprint time, respectively). There were no group x time interactions for RSI ($F_{2.2, 59.4}$ = 0.2, p = 0.816), MVC ($F_{2.8, 75.4}$ = 1.7, p = 0.172), and 30 m sprint time ($F_{4, 108}$ = 2.1, p = 0.086). However, a group x time interaction for CMJ ($F_{2.0, 53.8}$ = 3.5, p = 0.038) determined that the decline was greater in the SP group at 0 h (p = 0.001, d = 1.48) and recovery was greater at 72 h (p = 0.046, d = 0.81) in the DP group (where CMJ actually surpassed pre-exercise levels by 3.7%).

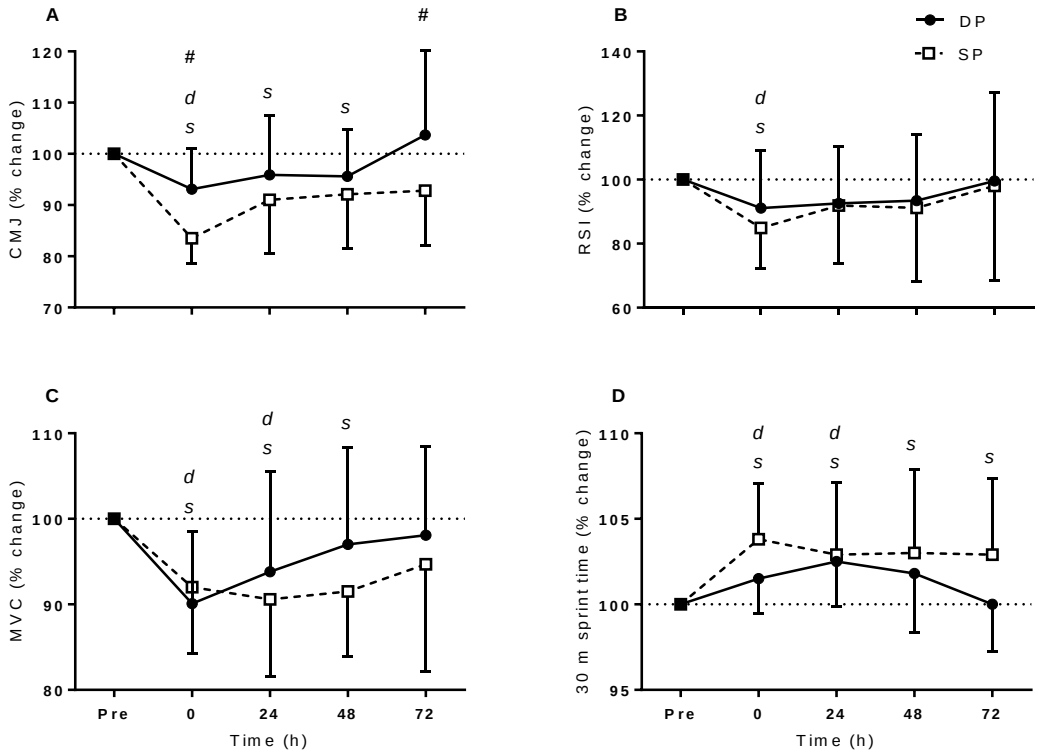

Figure 11. Percentage change from pre-exercise (pre) countermovement jump height (CMJ) (A), reactive strength index (RSI) (B), maximal voluntary isometric contraction (MVC) (C), and 30 m sprint time (D) following muscle-damaging exercise in the DP (*n* = 15) and SP (*n* = 14) groups. Values presented as mean ± SD. [d]denotes significantly different from pre-exercise in the DP group. [s]denotes significantly different from pre-exercise in the SP group. [#]denotes significant interaction effect. Significance at p < 0.05.

4.3.4 Creatine kinase

Pre-exercise concentrations of CK were 80.2 ± 39.5 vs 113.9 ± 57.6 IU·L^{-1} in the DP and SP groups, respectively (p = 0.108). Time effects were evident for CK ($F_{1.8, 40.5}$ = 17.2, p < 0.001). Both groups experienced an increase in circulating CK which peaked 24 h post-exercise (206.9 ± 143.9 vs 298.4 ± 158.9 IU·L^{-1} in the DP and SP groups, respectively) and remained elevated for 48 h in the DP group, and at all time-points post SP (Figure 12). Despite higher average CK levels in the SP group throughout testing, this was not significant; with no main effect of group (grand mean 163.0 ± 15.6 IU·L^{-1}, $F_{1, 22}$ = 3.1, p = 0.093) or group x time interactions ($F_{1.8, 40.5}$ = 0.7, p = 0.478).

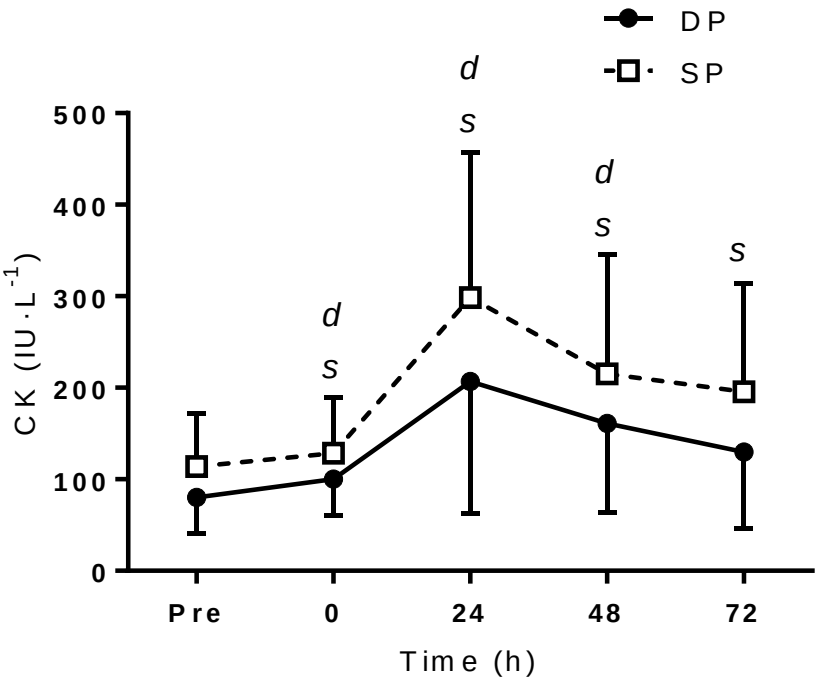

Figure 12. Total creatine kinase (CK) in response to muscle-damaging exercise in the DP (n = 12) and SP (n = 12) groups. Values presented as mean ± SD. [d]denotes significantly different from pre-exercise in the DP group. [s]denotes significantly different from pre-exercise in the SP group. Significance at p < 0.05.

Table 7. Values for dependent variables post muscle-damaging exercise, mean ± SD.

		Time post muscle-damaging exercise (h)				
Variable	**Group**	**Pre**	**0**	**24**	**48**	**72**
DOMS, mm	DP	6.0 ± 11.1	30.3 ± 28.1	90.1 ± 35.2	85.6 ± 41.8	39.1 ± 27.5
	SP	16.6 ± 36.0	47.8 ± 51.8	88.0 ± 47.4	59.1 ± 54.0	37.2 ± 44.6
Thigh girth, % (cm)	DP	100 ± 0	101.1 ± 1.0	100.5 ± 0.8	100.3 ± 0.9	100.1 ± 0.7
		(48.5 ± 3.1)	(49.1 ± 3.2)	(48.7 ± 3.3)	(48.7 ± 3.2)	(48.6 ± 3.2)
	SP	100 ± 0	100.2 ± 0.7	100.3 ± 0.8	100.3 ± 1.0	100.0 ± 0.7
		(48.5 ± 2.3)	(48.7 ± 2.4)	(48.7 ± 2.6)	(48.7 ± 2.6)	(48.5 ± 2.5)
Calf girth, % (cm)	DP	100 ± 0	99.7 ± 0.9	100.6 ± 0.5	100.4 ± 0.8	100.1 ± 0.9
		(36.0 ± 2.1)	(35.9 ± 2.2)	(36.2 ± 2.1)	(36.2 ± 2.2)	(36.1 ± 2.3)
	SP	100 ± 0	100.2 ± 0.8	100.3 ± 1.0	100.2 ± 0.8	100.0 ± 0.6
		(35.6 ± 1.7)	(35.7 ± 1.8)	(35.7 ± 1.6)	(35.7 ± 1.8)	(35.7 ± 1.8)
CMJ, % (cm)	DP	100 ± 0	93.1 ± 8.0	95.9 ± 11.5	95.6 ± 9.2	103.7 ± 16.6
		(26.3 ± 4.9)	(24.4 ± 4.8)	(25.0 ± 4.8)	(25.0 ± 4.5)	(26.9 ± 5.0)
	SP	100 ± 0	83.5 ± 4.8	91.0 ± 10.4	92.1 ± 10.6	92.7 ± 10.8
		(27.0 ± 4.1)	(22.3 ± 3.4)	(24.1 ± 4.4)	(24.4 ± 4.4)	(24.4 ± 4.0)
RSI, % (cm·s^{-1})	DP	100 ± 0	91.1 ± 18.1	92.6 ± 17.8	93.4 ± 20.6	99.6 ± 27.8
		(67.5 ± 24.2)	(59.5 ± 17.7)	(60.7 ± 18.4)	(61.5 ± 21.2)	(64.5 ± 20.8)
	SP	100 ± 0	84.9 ± 12.6	91.9 ± 18.2	91.2 ± 23.2	98.0 ± 29.4
		(72.4 ± 25.4)	(61.3 ± 23.4)	(66.2 ± 27.8)	(66.1 ± 30.3)	(69.2 ± 28.4)
MVC, %	DP	100 ± 0	90.1 ± 8.4	93.8 ± 11.7	97.0 ± 11.3	98.1 ± 10.4
(N)		(373.0 ± 62.8)	(335.7 ± 61.5)	(348.8 ± 65.1)	(361.7 ± 73.6)	(364.9 ± 66.5)
	SP	100 ± 0	92.0 ± 7.7	90.6 ± 9.0	91.5 ± 7.7	94.7 ± 12.5
		(382.9 ± 57.9)	(353.1 ± 64.6)	(347.4 ± 64.2)	(351.0 ± 63.5)	(363.6 ± 79.8)
30 m sprint time, %	DP	100 ± 0	101.5 ± 2.0	102.5 ± 2.6	101.8 ± 3.4	99.9 ± 2.8
(s)		(5.37 ± 0.37)	(5.44 ± 0.37)	(5.49 ± 0.32)	(5.46 ± 0.38)	(5.35 ± 0.29)
	SP	100 ± 0	103.8 ± 3.3	102.9 ± 4.2	103.0 ± 4.9	102.9 ± 4.5
		(5.27 ± 0.29)	(5.46 ± 0.19)	(5.41 ± 0.21)	(5.41 ± 0.20)	(5.41 ± 0.23)
CK, IU·L^{-1}	DP	80.2 ± 39.5	100.1 ± 40.3	206.9 ± 143.9	161.0 ± 97.6	129.8 ± 83.4
	SP	113.9 ± 57.6	128.7 ± 61.1	298.4 ± 158.9	215.1 ± 130.0	195.5 ± 118.6

DP, dance-specific protocol (n = 15); SP, repeated-sprint protocol (n = 14); pre, pre muscle-damaging exercise; DOMS, delayed onset muscle soreness; CMJ, countermovement jump height; RSI, reactive strength index; MVC, maximal voluntary isometric contraction; CK, creatine kinase (DP, n = 12; SP, n = 12).

4.4 Discussion

This investigation sought to examine the EIMD response and subsequent recovery following dance in female recreational dancers, and to gain a greater understanding of the consequences compared to more traditional muscle-damaging exercise. The first aim of this study was to ascertain a profile of EIMD indices following a dance-specific protocol. Results demonstrate that the DP successfully induced muscle damage with increases in DOMS, limb girth, plasma CK, and reductions in muscle function. These data agree with the extensive literature reporting that DOMS is evident soon after strenuous exercise, peaks at 24–48 h post-exercise, and remains elevated for several days (Armstrong, 1984; Cleak & Eston, 1992a; Proske & Morgan, 2001; Tee et al., 2007). Despite DOMS remaining significantly elevated for the duration of the study, the rise in soreness ratings were arguably relatively moderate compared to those reported following other eccentric exercise protocols (Howatson, Hoad, et al., 2012; Howatson et al., 2010). Lower limb muscles characteristically have a higher pain threshold (Fischer, 1987), and there is also evidence to suggest dancers have a high pain threshold due to the persistent musculoskeletal pain associated with dance participation (Ramel & Moritz, 1994). Aside from differences in muscle-damaging protocols employed in the literature, the combination of habituation to the level of soreness the participants are typically accustomed to, and the use of a predominantly lower limb muscle mass during the DP may explain the comparatively low perceived soreness levels.

While DOMS was significantly elevated for the duration of recovery, detriments in skeletal muscle function were not as substantial; with a return to near baseline levels of CMJ and RSI by 24 h and of MVC and 30 m sprint time by 48 h. Indeed, this study observed a normalised loss in CMJ of just 6.9% immediately post DP. Although this decrement is comparable to that reported in a recent study following intermittent running (Leeder et al., 2014), others have demonstrated considerably greater losses (> 20%) in CMJ (Garcia-Lopez et al., 2006) and MVC (Goodall & Howatson, 2008; Howatson & Milak, 2009) following heavy, eccentric-biased protocols. These inconsistencies are almost certainly attributable to the distinct differences in exercise stress, notably the intensity and nature of the damaging

protocol adopted. Moreover, these activities are frequently encountered during training and performance in the present study population. Jumps and landing tasks are incorporated in most dance activities (Paschalis et al., 2012) and many dance movements are characterised by explosive actions (Westblad et al., 1995). Conceivably, the recreational dancers recruited in the present investigation were probably accustomed, to some extent, to the dance-specific nature of the protocol and had some degree of protection precipitated by the repeated bout effect (Howatson et al., 2007; McHugh, 2003; Nosaka, Sakamoto, Newton, & Sacco, 2001). Thus, the training status of participants in this study may explain the relatively rapid return to baseline levels of measures of muscle function following the DP.

Sex differences may also account for some of the discrepancies in the literature. To date, the majority of research investigating EIMD has used male volunteers and the potential differences across the sexes are largely overlooked. There is evidence to suggest that oestrogen may have a protective effect against EIMD with reported characteristics including membrane stabilising properties (Tiidus, 2000). This potential attenuation of membrane disruption may account for some of the steroid hormone's mitigating effects on structural and mechanical damage; and therefore its part in attenuating declines in muscle function on subsequent days. In addition, the suggested oestrogenic influence over membrane permeability may also explain the low CK values observed in the current study in comparison to previous research; where values have reached in to the high hundreds or thousands (Cooke et al., 2010; Howatson et al., 2010; Howatson & Milak, 2009; Leeder et al., 2014). Nevertheless, the raised CK extending for several days is reflective of a damaging bout of exercise (Mougios, 2007). Moreover, these data are comparable to those reported during recovery in elite female dancers (Rodrigues-Krause et al., 2014), despite the difference in exercise stimulus and participant training status. The intensity of the exercise in their study was reported to be 95% HR_{max} and 66.2% $\dot{V}O_{2max}$ of the elite dancers during rehearsal (Rodrigues-Krause et al., 2014). Conversely, the study which developed and validated the original DP observed values of 90% HR_{max} and $\dot{V}O_2$ values around $\dot{V}O_{2max}$ during the DP (Redding et al., 2009). Given the intensity of the protocol reported in the original investigation and the lower training status of the dancers in the current study, a greater muscle damage response might have been

expected. Nevertheless, the DP is representative of an activity-specific stimulus that is encountered during dance performance. Moreover, the present study is in agreement with a number of investigations reporting that trained female participants experience EIMD, despite their familiarity with the damaging exercise stimulus (Baur, Bach, Hyder, & Ormsbee, 2016; Clarke, Anson, & Pyne, 2015; Ferreira et al., 2016).

The second aim of this investigation was to determine whether the magnitude of damage experienced following dance-specific exercise (and its consequences) is comparable to that of a more traditional sport-specific exercise model. The SP represents the muscle damage response that might follow field sport activity such as soccer, rugby and field hockey. The profile of damage following the SP in the present study was similar to that observed in previous work with male (Howatson & Milak, 2009) and female (Keane, Salicki, et al., 2015) participants, although the magnitude of damage appeared less severe in the current study. However, as anticipated, increases in DOMS, circulating CK and limb girth, and reductions in muscle function post-exercise persisted for several days. Interestingly, the decrements in measures of muscle function in both groups were greatest immediately post-exercise, with the exception of MVC following SP and 30 m sprint time following DP which reached lowest levels 24 h post-exercise. These responses were surprising given that the combination of both muscle damage and metabolic fatigue immediately post-exercise could result in the greatest decline of neuromuscular function, as evidenced by the other functional measures. Perhaps the different exercise protocols employed in the literature are responsible for this discrepancy. While the muscles may have been put under maximum stress in the present investigation, the time under tension was far less than during traditional, isolated muscle contraction models. As a result, the fatigue component might be less evident in these sport-specific protocols.

There were no group differences in dependent variables between the DP and SP; demonstrating that muscle damage and recovery was comparable between groups. However, though changes in variables did not differ significantly between groups, the pattern of recovery was not the same. Both limb girths increased following the DP, but not post SP, and an interaction effect was observed in thigh girth immediately following EIMD. Intuitively, this might suggest a greater blood flow

and/or secondary inflammatory response associated with the dance-type activity. Indeed, it is likely that the differences in the nature and demands of the exercise protocols are responsible for the variance in muscle swelling. However, since no other measures of inflammation were taken, this remains to be elucidated. In addition, while all measures had returned to near pre-exercise muscle function by 48 h or sooner in the DP group, the SP group appeared to experience a greater magnitude of impairment and seemed to recover less quickly; notably in skeletal muscle function (including interaction effects for CMJ). Repetitive jump protocols (Goodall & Howatson, 2008; Howatson, Hoad, et al., 2012; Jakeman, Macrae, & Eston, 2009), which form part of the DP, have been shown to elicit muscle damage. However, it is possible that the substantial eccentric demand of the accelerations and decelerations in the SP may provide a greater stimulus for mechanical disruption to the myofilaments in skeletal muscle. Moreover, though a homogenous population was recruited in order to better control for training status and free-living physical activity levels and dietary behaviours, the participants were recruited from a university dance team. While dance-type exercise might include elements of sprint activity (Cohen et al., 1982), the SP could be considered a novel exercise stimulus for the study population, which is likely to have exacerbated the damage response (Howatson & van Someren, 2008). Nevertheless, this is the first study to demonstrate that both dance-specific and sport-specific exercise elicits EIMD in female dancers and despite some small variations in the physiological profiles following damage and during recovery, there were no group differences between these exercise paradigms.

4.5 Perspectives

This chapter addressed the second aim of the thesis: *'to examine the exercise-induced muscle damage response to both dance-specific and sport-specific exercise in female dancers'*. The results from this study resulted in the rejection of the null hypothesis, concluding that there were no significant differences in the exercise-induced muscle damage response following both dance-specific and repeated-sprint exercise in female dancers. The findings demonstrated that EIMD is experienced following dance-type exercise and the associated symptoms persist for several days.

Specifically, these data substantiate the previous work indicating that dance activity increases systemic indices of damage, and demonstrated that this occurs with concomitant increases in muscle soreness, and reductions in muscle function. Perhaps the most interesting finding was that, while there were interaction effects for thigh girth and CMJ, the magnitude of damage and time-course of recovery post DP was similar to a more traditional SP protocol. Thus, this investigation lends support for the efficacy of both the DP as a model to induce muscle damage in a dance-specific manner, and the use of the SP in this population as recovery from exercise stimuli of this nature was similar. These results add to a growing body of evidence demonstrating that different exercise paradigms can elicit EIMD. Importantly, this study offers practical applications for both applied sport scientists and female dancers alike, by identifying, and enhancing understanding of a number of implications associated with engaging in strenuous, potentially damaging exercise. However, beyond the measured effects of this study, there may be other repercussions following EIMD which could affect performance potential in this population, including (but not limited to) reduced joint position sense and reaction time, and increased risk of injury. These data therefore provide rationale for the investigation of potential interventions, which could attenuate the associated negative symptoms of EIMD in female dancers.

To date, this course of investigation has identified that female dancers are at risk of both energy deficiency and muscle damage. Consequently, the following experimental chapters 5 and 6 sought to address these issues by investigating the efficacy of nutritional interventions on recovery from EIMD in female dancers. Given that this study had identified that both dance-specific and repeated-sprint exercise protocols elicited a similar damage and recovery response, subsequent studies employed the SP protocol as an appropriate model to induce muscle damage in this population.

5 Montmorency tart cherry (*Prunus cerasus* L.) supplementation and exercise-induced muscle damage in female dancers

5.1 Introduction

Antioxidant supplementation is of considerable interest to research in clinical populations given the role of these bioactive compounds in reducing oxidative stress and inflammation. Numerous interventional strategies have been cited to benefit inflammatory-related diseases, including products of beetroot (Martinez et al., 2015; Pietrzkowski et al., 2010), cranberry juice (Bodet, Chandad, & Grenier, 2006; Duffey & Sutherland, 2015), blueberries (Pervin et al., 2016; Zhong et al., 2015), and sweet cherries (Jacob et al., 2003; Kelley, Rasooly, Jacob, Kader, & Mackey, 2006). Tart cherries have also demonstrated efficacy in inflammatory conditions such as osteoarthritis (Kuehl, Elliot, Sleigh, & Smith, 2012; Schumacher et al., 2013), as well as in the management of fibromyalgia (Elliott, Kuehl, Jones, & Dulacki, 2010), sleep (Howatson, Bell, et al., 2012; Pigeon, Carr, Gorman, & Perlis, 2010) and in reducing blood pressure in pre-hypertensive males (Keane et al., 2016).

The growing research indicating potential benefits and applications of cherries in clinical groups also has relevance to exercising populations. During exercise, initial muscle damage is thought to be caused by a combination of mechanical disruption to the myofibrils and oxidative stress; the latter owing to an increase in the production of reactive oxygen and nitrogen species (RONS) and nitric oxide (NO) derivatives which may exceed antioxidant capacity (Powers & Jackson, 2008). Moreover, the secondary inflammatory response to muscle injury involves the degradation of damaged muscle by immune cells which release pro-inflammatory cytokines and further RONS and NO derivatives; exacerbating muscle damage (Clarkson & Hubal, 2002). Indeed, the role of RONS and NO derivatives in the oxidative stress, inflammation, and muscle damage responses which manifest during and following exercise has raised substantial interest in antioxidant supplementation. Of particular interest is tart Montmorency cherries (MC), which has been proposed to be an effective recovery aid due to the high anti-inflammatory properties and antioxidant content present within it (Bell, Walshe, et al., 2014; Bell et al., 2015; Keane, Bell, et al., 2015; Kirakosyan et al., 2015; Seeram et al., 2001; Wang, Nair, Strasburg, Chang, et al., 1999). While sweet cherries are thought to contain greater amounts of anthocyanins (Chaovanalikit & Wrolstad, 2004; McCune et al., 2011), sour cherries (such as the Montmorency cultivar) have been shown to contain

greater amounts of total phenolics (Chaovanalikit & Wrolstad, 2004; Ferretti, Bacchetti, Belleggia, & Neri, 2010; Kim, Heo, Kim, Yang, & Lee, 2005), and these appear to be highest (per serving) in MC juice concentrate when compared to frozen, canned or dried MC (Keane, Bell, et al., 2015; Ou et al., 2012). Moreover, the polyphenolic compounds that MC contain result in higher ORAC values compared to several other antioxidant beverages such as Concord grape, acai, iced green tea, and blueberry juice (Bell et al., 2013; Howatson et al., 2010; Seeram et al., 2008). The roles of individual phytochemicals following strenuous exercise are not fully understood, however it is likely that they act in synergy to provide a positive influence against the symptoms associated with strenuous exercise.

To date, research in exercise and recovery paradigms has demonstrated that MC can improve recovery from damaging bouts of exercise in isolated muscle groups by attenuating decrements in muscle strength and/or soreness and pain (Bowtell et al., 2011; Connolly et al., 2006; Levers et al., 2015). Additionally, following damaging running activity, research has identified MC to be beneficial in reducing pain (Kuehl et al., 2010) and reducing indices of inflammation and oxidative stress, and increasing antioxidant status and muscle function (Howatson et al., 2010). MC has also been shown to facilitate recovery using an exercise model in which oxidative stress and inflammation is induced exclusively via metabolic pathways (cycling) as opposed to mechanically-induced exercise stress through eccentric muscular contractions (Bell, Walshe, et al., 2014; Bell et al., 2015). More recently, a reduction in inflammation and muscle soreness, as well as an increase in muscle function has been observed with MC following an adapted Loughborough Intermittent Shuttle Test (LIST; a strenuous repeated, intermittent-sprint exercise protocol which includes 5 sets of approximately 15 mins of varying-intensity exercise) (Bell et al., 2016).

Collectively, these lines of investigation have application to athletic populations that participate in strenuous activity that precipitates detrimental consequences to performance in the days following the exercise insult. However, knowledge of the effects of MC beyond isolated muscle, running and cycling activity are limited and conceptually other sports and activities where exercise recovery can be identified as an issue could benefit from this intervention. An intervention that can manage the negative consequences of other types of physical activity would present a wider

application of its use. For instance, dance activity has been shown to result in oxidative stress and muscle damage (Rodrigues-Krause et al., 2014), and may therefore benefit from an antioxidant supplementation strategy. In addition, there are no data regarding exclusively female populations, largely due to the potential to confound results because of monthly variability in the female sex hormones (namely oestrogen) that might influence outcome variables. Though some studies have included female participants (Howatson et al., 2010; Kuehl et al., 2010), mixed sex treatment groups fail to acknowledge the potential influence of sex in the muscle damage and recovery responses. Therefore, it is clear that more research investigating the influence of nutritional interventions using single sex groups is essential in order to develop our understanding; namely in females where information is scarce.

Though contradictory findings are evident in the literature, MC supplementation has shown some efficacy in exercise recovery consistently across various laboratories (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010; Kuehl et al., 2010; Levers et al., 2015). However, whether these benefits are also shown following exercise in female dancers remains to be elucidated. Therefore, the aim of this investigation was to examine the efficacy of a MC concentrate on recovery following a bout of exercise designed to cause temporary muscle damage in a population of female dancers. It was hypothesised that indices of EIMD and inflammation would be attenuated by the consumption of MC. Consequently, this chapter sought to address the third aim of the thesis: *'to investigate the influence of Montmorency tart cherry juice supplementation on exercise-induced muscle damage in female dancers'*.

5.2 Materials and methods

5.2.1 Participants

5.2.1.1 Recruitment

Please refer to section 4.2.1.1 for details of recruitment strategy, as well as exclusion criteria.

5.2.1.2 Sample size

Please refer to section 4.2.1.2 for details regarding the rationale for use of MVC to calculate sample size. The sample size was determined by completing a power analysis (power = 0.8, $\alpha = 0.05$) based on isometric strength data from Bowtell et al. (2011). This determined a sample size of five in each group would provide statistical power above 80%, with an alpha level of 0.05. In order to account for dropouts, the aim was to recruit a sample of 10 per group.

5.2.1.3 Participant characteristics

Twenty healthy recreationally active females (mean ± SD age 19 ± 1 y; stature 166.7 ± 5.5 cm; body mass 61.4 ± 5.7 kg; and BMI 22.1 ± 1.9 $kg \cdot m^{-2}$, respectively) were recruited from a university dance team and gave written informed consent (Appendix A). Participant characteristics in both treatment groups are presented in Table 8. Participants recorded their exercise behaviours and completed a weighed food diary throughout the supplementation and trial periods (7 days; 4-day preload period, day of exercise, and two days post exercise) in order to establish energy and macronutrient intake. In addition, portions of foods thought to contain antioxidants were totalled for each day and averaged across the experimental period (Howatson, Bell, et al., 2012; Howatson et al., 2010). The study was conducted according to the guidelines of the Declaration of Helsinki and all experimental procedures were approved by the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria (HLSMB130715).

Table 8. Participant characteristics, mean ± SD.

	MC (*n* = 10)	PL (*n* = 10)	*p* value[1]
Age (y)	19 ± 1	20 ± 1	0.538
Body mass (kg)	61.1 ± 5.7	61.7 ± 6.1	0.837
Stature (cm)	167.6 ± 4.2	165.7 ± 6.7	0.457
BMI ($kg \cdot m^{-2}$)	21.8 ± 2.3	22.4 ± 1.5	0.455
Dance training (y)	13 ± 5	14 ± 3	0.567
Dance training ($h \cdot week^{-1}$)	6.2 ± 1.8	5.6 ± 0.8	0.350
Total exercise ($h \cdot week^{-1}$)	8.9 ± 7.7	7.0 ± 1.9	0.598

[1]Tart Montmorency cherry (MC) vs placebo (PL) compared by independent samples *t* test.

5.2.1.4 Dietary and exercise restrictions

For 24 h prior to, and for each of the testing days, participants were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements (including vitamin and mineral tablets), and any anti-inflammatory drugs or alternative treatments (including massage and cold water immersion). For more details please refer to section 4.2.1.4. These restrictions were employed to limit the influence of diet and physical activity on the dependent variables and ensured that observed effects were likely to be in response to the supplementation implemented within the study.

5.2.2 Pre-testing procedures

A menstrual cycle questionnaire (Appendix C) identified the contraceptive use of participants; nine were using an oral combination pill (all monophasic), six were using a progesterone only pill/implant/injection, and five were menstruating normally. This also determined menstrual cycle phase; all data collection took place during the early to mid-luteal phase, or where applicable in the 14 days before a withdrawal bleed. Participants were initially required to attend the laboratory for familiarisation with the procedures and the exercise protocol was described. Finally, participants were required to complete three maximal voluntary isometric

contractions (MVC) of the knee extensors (described in detail in section 4.2.5.3.3); the peak of which was used for stratified randomisation. For more details regarding these pre-testing procedures (~1-2 weeks prior to data collection), please refer to section 4.2.2. In addition to these procedures, a pre-supplementation blood sample (baseline) was taken in order to detect any changes in systemic indices with preload supplementation, prior to subsequent laboratory visits. Participants were then required to attend the laboratory on four further occasions, each following an overnight fast. Participants were tested at the same time on subsequent days (± 1 h) to account for diurnal variation.

5.2.3 Experimental protocol

This study adopted an independent groups design and used stratified randomisation (described in section 4.2.2) to assign participants to one of two groups; tart Montmorency cherry juice (MC) or a placebo (PL). The study was conducted in a double-blind, placebo-controlled manner. Following written informed consent and pre-testing procedures (described in section 4.2.2), participants attended the laboratory for a further four consecutive days. Participants were fasted for ≥10 h prior to each laboratory visit, except for water (which was consumed *ad libitum*) and the morning supplement, which was consumed 1-2 h prior to each visit. On arrival at the laboratory participant characteristics (stature measured to the nearest 1 mm (stretch stature technique, Model 220, Seca Ltd, Birmingham, UK) and body mass to the nearest 0.1 kg (Model 876, Seca Ltd, Birmingham, UK)) and baseline measures of dependent variables were recorded. On completion of these baseline measures participants completed the exercise protocol designed to induce muscle damage. After a 2 min rest, measurement of dependent variables was repeated. Before leaving the laboratory, participants were reminded to continue the supplementation strategy and consume a supplement prior to their evening meal. Supplementation and measurement of dependent variables were then repeated following an overnight fast and in the same order for the following 3 days after the exercise (24, 48 and 72 h post EIMD). Please refer to Figure 13 below for an illustration of the study design, and the following sections for details regarding all data collection procedures. Protocols and measurement of dependent variables were completed in

an indoor sporting facility and environmental conditions were controlled (temperature, 19.8 ± 1.4^{0}C; pressure, 1011.3 ± 12.4 hpa; humidity, 48.4 ± 8.3%).

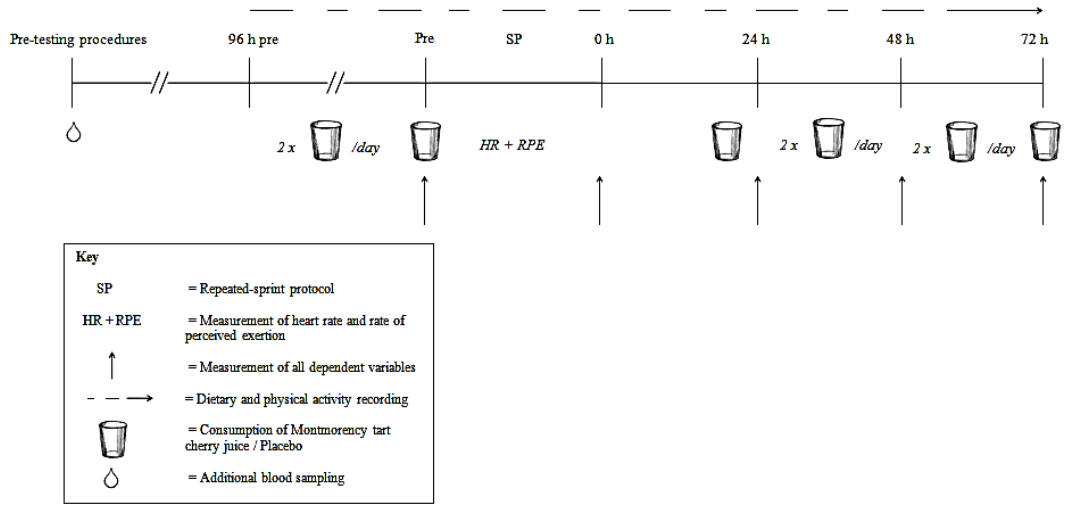

Figure 13. Schematic of testing protocol.

5.2.4 Supplementation

Participants were provided with eight days of supplementation along with instructions on ingestion frequency and timing. This period was for four days prior to muscle-damaging exercise, the day of exercise, and for three days of recovery. The daily dose was two servings of the MC or PL; one dose taken prior to breakfast (or 1-2 h prior to laboratory visits), and one dose prior to evening meal (except for the final day where only one supplement was consumed before the final visit). This is based on previous work showing a positive effect on recovery following strenuous exercise (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015). Anthocyanin bioavailability in plasma and urine has been typically reported to peak 1-2.5 h post ingestion despite differences in dosage volume (for review please refer to Manach et al. (2005)). Specifically, phenolic compounds in the same MC product used in the current study were previously shown by our laboratory to be most abundant in plasma in the 1-2 h post consumption (Keane, Bell, et al., 2015). Moreover, anthocyanin metabolites have been reported to be present in urine 24 h post ingestion (Felgines et al., 2003) and up to 48 h post consumption in faeces (Czank et al., 2013). Therefore, the supplementation strategy allows for optimal concentrations

both coinciding with the laboratory visits and in the hours or days post-exercise when inflammation and oxidative stress are thought to persist.

Previously, it has been shown that in healthy adults, overall abundancy of phenolic compounds detected in plasma (Keane, Bell, et al., 2015), and changes in urinary and serum urate and serum hsCRP are not different between 30 mL and 60 mL doses of MC concentrate (Bell, Gaze, et al., 2014). Therefore, the MC beverage was prepared with 30 mL of concentrate (CherryActive, Sunbury, UK) diluted in 100 mL of water. According to the manufacturer's information, a 30 mL dose of concentrate is equivalent to approximately 90 whole cherries and has been previously reported to contain 9.117 $mg \cdot mL^{-1}$ of anthocyanins (Bell et al., 2013). Moreover, independent analysis of the MC (Atlas Biosciences Analytical Report; Atlas Biosciences: Tucson, AZ, USA, 2010) has detailed further compositional information; fat 0.028 $mg \cdot mL^{-1}$, protein 31.47 $mg \cdot mL^{-1}$, carbohydrate 669.4 $mg \cdot mL^{-1}$, cholesterol < 0.01 $mg \cdot mL^{-1}$, sodium 0.691 $mg \cdot mL^{-1}$, calcium 0.137 $mg \cdot mL^{-1}$ and iron 0.026 $mg \cdot mL^{-1}$. The PL was prepared with 25 mL of a synthetically derived fruit flavoured concentrate with negligible phytochemical content (Kia-Ora, Uxbridge, UK) in 100 mL of water and was fortified with flavourless maltodextrin (Myprotein, Manchester, UK) and flavourless whey protein powder (Arla Foods, Amba, Denmark). This was in order to match test beverages as closely as possible for volume, consistency, colour, and macronutrient and energy content. Participants were asked to keep beverages in a cool and dark place (preferably refrigerated at 4^0C) in order to minimise the potential degradation of bioactive compounds by light and heat. Test beverages were prepared by the principal investigator and were labelled in a double-blind manner in masked bottles (by an individual not directly involved in the research). Following all data collection periods, only $n = 4$ participants correctly identified which supplement they had consumed. Please refer to Table 9 for the composition of beverages.

Table 9. Composition of test beverages.

	MC	PL
Energy		
(kcal)	102	103
(KJ)	427	431
Volume (mL)		
Total	130	130[1]
Water	100	100
Concentrate	30	25
Total carbohydrate (fortified) (g)	24.5	24.5 (24.13)
Total protein (fortified) (g)	1.1	1.1 (1.1)
Fat (g)	0	0

[1]Volume corresponded to 130 mL when 125 mL liquid was fortified with maltodextrin (Myprotein, Manchester, UK) and protein powder (Arla Foods, Amba, Denmark). Tart Montmorency cherry (MC) and placebo (PL) beverages.

5.2.5 Exercise protocol

Prior to baseline measurement of muscle function and prior to the exercise protocol, participants completed a standardised warm up. This consisted of 5 minute treadmill running at a self-selected pace to induce a rate of perceived exertion (RPE) (Borg, 1982) of 11-12 (described in section 5.2.5.1). Participants were also given 5 minutes to perform any personal stretches and prepare themselves for measurement of muscle function and the assigned protocol. Each participant's individual warm up on the initial day was noted so this could be replicated throughout testing. Standardised instructions and strong verbal encouragement from the investigator to encourage maximal effort were provided throughout the muscle-damaging protocol. Participants completed the SP, which briefly involves 15 x 30 m sprints separated by 60 s rest to induce muscle damage. Please refer to section 4.2.4.3 for details regarding this protocol. RPE and HR were also collected after each sprint effort to determine exercise intensity; these are described in sections 5.2.5.1 and 5.2.5.2.

Given that study 2 identified that both dance-specific (DP) and repeated-sprint (SP) exercise protocols elicited a similar damage and recovery response, the SP protocol was considered an appropriate model to induce muscle damage in this population. This protocol is not associated with many of the logistical limitations associated with the DP (not least in the requirement of participants to have learnt the dance

sequence and the necessity for adequate and appropriate space) and is a more feasible protocol when considering time constraints associated with this work. Moreover, previous work has shown centre floor exercise and stage performance in dance includes sprint-like and power related tasks (Cohen et al., 1982), and the intermittent nature of the SP is also analogous with contemporary dance performance (Wyon, 2005; Wyon et al., 2002). Please refer to section 7.3 for further discussion on the rationale for the use of SP.

5.2.5.1 Rate of perceived exertion (RPE)

The Borg scale was administered to assess ratings of perceived exertion (RPE) after each sprint effort during the SP (Borg, 1982). The scale included written descriptors anchored to numbers that relate to psycho-physiological perceptions of effort. This ranged from 6 representative of 'very very light' exercise, and 20 representative of 'exhaustion'. This scale has been found to successfully monitor effort perception and is comparable to objective measures of physical exertion (Chen, Fan, & Moe, 2002; Skatrud-Mickelson, Benson, Hannon, & Askew, 2011). Participants were asked to define their level of perceived exertion after each sprint during the SP. Average and peak RPE was used to demonstrate exercise intensity.

5.2.5.2 Heart rate (HR)

Participant HR was monitored by portable heart rate telemetry (Model RS-400, Polar, Kempele, Finland) to determine exercise intensity. HR has been shown to be an accurate field-based method for assessing exercise intensity (Wallace, Slattery, Impellizzeri, & Coutts, 2014) and is moderately correlated with $\dot{V}O_2$ (Strath et al., 2000). Participants were required to wear a transmitter strap around their chest and a watch-like receiver on their wrist. HR was recorded immediately after each sprint effort during the SP. Average and peak HR was used to demonstrate exercise intensity.

5.2.6 Dependent variables

The following dependent variables were measured pre, immediately post (0 h), and 24, 48, and 72 h post muscle-damaging exercise. An additional blood sample was also taken prior to supplementation (baseline).

5.2.6.1 Muscle soreness

5.2.6.1.1 Active muscle soreness (DOMS)

Muscle soreness was assessed subjectively using a VAS. Please refer to section 4.2.5.1 for detailed information.

5.2.6.1.2 Pain pressure threshold

Pressure algometry was also used to objectively measure muscle soreness. Algometry traditionally measures pain pressure threshold (PPT) of a given location (Fischer, 1987). PPT has been used to monitor symptoms of experimental delayed onset muscle soreness and pain following EIMD in a number of studies (Clifford et al., 2016; Connolly et al., 2006; Levers et al., 2015; Peschek et al., 2014). It has been shown to be a reliable measure, and repeated algometry over consecutive days does not appear to alter PPT (Nussbaum & Downes, 1998). PPT in lower extremity muscle groups was measured with a digital algometer with a connecting 1.0 cm^2 flat, circular rubber disc (Model FDX, Wagner Instruments, Greenwich, USA). Three muscle locations were determined; the rectus femoris (RF), the vastus lateralis (VL), and medial head of the gastrocnemius (GM) (Clifford et al., 2016). The RF was located at the mid-thigh; the midpoint between the inguinal fold and the superior border of the patella. The VL was located at the midpoint between the superior point on the greater trochanter and the superior point on the lateral border of the head of the tibia where the muscle is at its greatest thickness. The GM location was determined as the site of the most medial aspect of the calf at the level of relaxed maximal girth. All measurements were taken on the right side of the participant and were marked with permanent marker to ensure accuracy on consecutive days (Vatine, Shapira, Magora, Adler, & Magora, 1993). The algometer was applied

perpendicular to the body surface while supine, and the pressure was applied at an approximate rate of 5 $N \cdot s^{-1}$ to increase reliability. To determine PPT, participants were asked to verbally indicate when the force became uncomfortable. The PPT at each location was measured twice at each time point and if a difference of ± 5% was observed then a third measure was taken. The average of the two closest measurements was used for statistical analysis. Intra-trial and inter-trial percentage coefficient of variation (%CV) was established from reliability testing at < 8% and < 5% respectively at the RF, < 5% and < 4% respectively at the VL, and < 7% and < 8% respectively at the GM.

5.2.6.2 Limb girth

Thigh and calf girth were measured to assess inflammatory swelling and oedema. Please refer to section 4.2.5.2 for detailed information.

5.2.6.3 Hamstring stiffness and flexibility

Hamstring stiffness and flexibility were measured using the sit and reach test. Sit and reach tests have been used extensively in the literature and have been demonstrated to be a moderately valid indirect measure of hamstring and low back flexibility (Baltaci, Un, Tunay, Besler, & Gerceker, 2003; Hui & Yuen, 2000). Please refer to section 2.2.2.1 for more details regarding the use of this marker following EIMD, and its importance to dance populations. Participants were required to sit with their knees fully extended and feet together against the sit and reach box; the heel position in line with the 15 cm position on the box. With one hand placed over the other, participants were instructed to slowly reach forward along the measuring board to avoid rapid or forceful movements. They were asked to stretch as far as possible (but not to the point of pain) and to hold their 'best stretch' for approximately 3 s. The score of this final position was recorded to the nearest 0.5 cm. To account for the advantage offered to an individual with a long trunk, long arms, and short legs (Broer & Galles, 1958; Wear, 1963), these scores were analysed as the percentage change relative to baseline measurement. Intra-trial and inter-trial %CV was established from reliability testing at < 5% and < 3% respectively.

5.2.6.4 Muscle function

Muscle function was assessed via countermovement jump height (CMJ), reactive strength index (RSI), maximal voluntary isometric contraction of the knee extensors (MVC) and 30 m sprint time. Please refer to section 4.2.5.3 for detailed information.

5.2.6.5 Blood sampling and analysis

Blood samples (10 mL) were collected via venepuncture from the antecubital fossa area into serum gel vacutainers (Vacutainer BD UK Ltd, Oxford, UK). After allowing samples to rest and clot at room temperature for a minimum of 20 min, samples were centrifuged for 15 min (4˚C) at 3000 RCF in order to obtain serum. The aliquots were stored at -80°C for later analysis of total CK and hsCRP. Due to difficulties with blood sampling, where data for a single time point were missing (5 points were missing out of a total of 120 (< 5%) for each blood-based variable), the group mean was used to complete the data set.

5.2.6.6 Total creatine kinase analysis

Serum total CK concentrations were determined spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, Burgess Hill, UK). Please refer to section 4.2.5.4.1 for detailed information.

5.2.6.7 High-sensitivity C-reactive protein (hsCRP) analysis

Systemic hsCRP is a sensitive and accurate marker of systemic inflammation (Pepys & Hirschfield, 2003). Please refer to sections 2.2.2.3 and 2.3.1.2 for more details regarding the use of this marker following EIMD, and its specific relevance to the potential actions of MC. Serum hsCRP concentrations were determined spectrophotometrically (Roche Modular, Roche Diagnostics, Burgess Hill, UK) using a particle enhanced immunoturbidimetric assay. Human hsCRP agglutinates with latex particles were coated with monoclonal anti-hsCRP antibodies and the turbidity was measured at 546 nm. The measurement range for this method was 0.15-20.0 $mg \cdot L^{-1}$ and normal reference values are 1.0-3.0 $mg \cdot L^{-1}$. When lower

detection limits were not reached, the lowest detectable concentration was used (0.15 $mg \cdot L^{-1}$). The inter-assay and intra-assay %CV were < 9% and < 3% respectively.

5.2.7 Statistical analysis

Previously described statistical analysis methods were employed (please refer to section 4.2.6). Results are presented as means ± SD. For the purpose of data analysis, all dependent variables except for DOMS, CK and hsCRP are expressed as a percentage change relative to pre muscle damage values to account for inter-individual variability. Statistical software (IBM SPSS V22, IBM, Armonk, USA) was used for inferential analysis and statistical significance was accepted at the $p \leq 0.05$ level *a priori*. Two-way group (2; MC vs PL) x time (5; pre, and 0, 24, 48 and 72 h post EIMD) repeated measures analysis of variance (ANOVA) were performed for each dependent variable to assess for differences in group and time. Significant main effects were analysed using the Least Significant Difference test (LSD) for adjustment for multiple comparisons. Paired samples *t* tests were conducted to assess differences between total CK and hsCRP levels pre-supplementation (baseline) and pre-exercise, in order to detect any changes in systemic indices with preload supplementation. Independent samples *t* tests were conducted on peak HR, peak RPE, fatigue, and total and mean sprint time to examine differences in exercise intensity during the SP between groups. Where appropriate, Cohen's D effect sizes (*d*) were calculated with the magnitude of effects considered small (0.2), medium (0.5) and large (> 0.8).

5.3 Results

All sampling distributions were considered normally distributed. There were no group differences in absolute pre-exercise values of all dependent variables, except PPT at all three locations, flexibility, and RSI (independent samples *t* test, $p < 0.05$); where these were higher in the MC group. However, prior to data analysis, these variables were expressed as a percentage change relative to pre muscle damage values to account for inter-individual variability. There were no differences in the

total energy intake, macronutrient intake, and the number of portions of foods containing antioxidants consumed by participants in both treatment groups during the supplementation period (data presented in Table 10). There were no differences (all $p > 0.05$) between MC and PL groups for total sprint time (80.74 ± 4.02 vs 81.69 ± 3.67 s), mean sprint time (5.38 ± 0.27 vs 5.45 ± 0.24 s), fatigue (5.23 ± 2.02 vs 4.54 ± 2.16%), peak HR (176 ± 15 vs 178 ± 8 bpm), and peak RPE (17 ± 2 vs 18 ± 1) during the SP protocol; demonstrating that exercise stimulus was comparable between groups. Time effects were observed for all dependent variables ($p < 0.05$), except limb girth and hsCRP, which demonstrated the presence of EIMD. All dependent variable data are presented in Table 10.

Table 10. Daily dietary intakes[1], mean ± SD.

Variable		MC	PL	*p* value[2]
Energy	kcal	1688 ± 470	1428 ± 383	0.192
	MJ	7.1 ± 2.0	6.0 ± 1.6	0.192
Carbohydrate	$g \cdot kg^{-1}$	3.8 ± 1.5	2.9 ± 0.9	0.125
	%TEI	53 ± 7	50 ± 5	0.274
Protein	$g \cdot kg^{-1}$	1.1 ± 0.3	0.9 ± 0.3	0.137
	%TEI	16 ± 3	15 ± 1	0.203
Fat	$g \cdot kg^{-1}$	1.0 ± 0.3	1.0 ± 0.4	0.829
	%TEI	33 ± 5	37 ± 5	0.056
Portions of food containing antioxidants per day		6 ± 2	6 ± 2	0.731

[1]Excluding supplementation; as determined using dietary analysis software (Nutritics Ltd, Swords, Ireland) from a 7-day weighed food diary completed during the supplementation period. %TEI, percentage of total energy intake.[2]MC, tart Montmorency cherry group (n = 10) vs PL, placebo (n = 10) compared by independent samples t test.

5.3.1 Muscle soreness

Pre-exercise DOMS was 9.7 ± 14.2 vs 21.3 ± 25.5 mm in the MC and PL groups, respectively (p = 0.225). There was a main effect of time for DOMS ($F_{2.7, 48.8}$ = 21.3, p < 0.001). Muscle soreness increased immediately post-exercise, peaking at 24 h post-exercise (54.8 ± 35.3 vs 87.4 ± 40.0 mm in the MC and PL groups, respectively). DOMS remained elevated throughout recovery in both groups; however, there was a trend for lower DOMS in the MC group ($F_{1, 18}$ = 3.7, p = 0.070, d = 0.58) (Figure 14). There were no interaction effects ($F_{2.7, 48.8}$ = 1.1, p = 0.358).

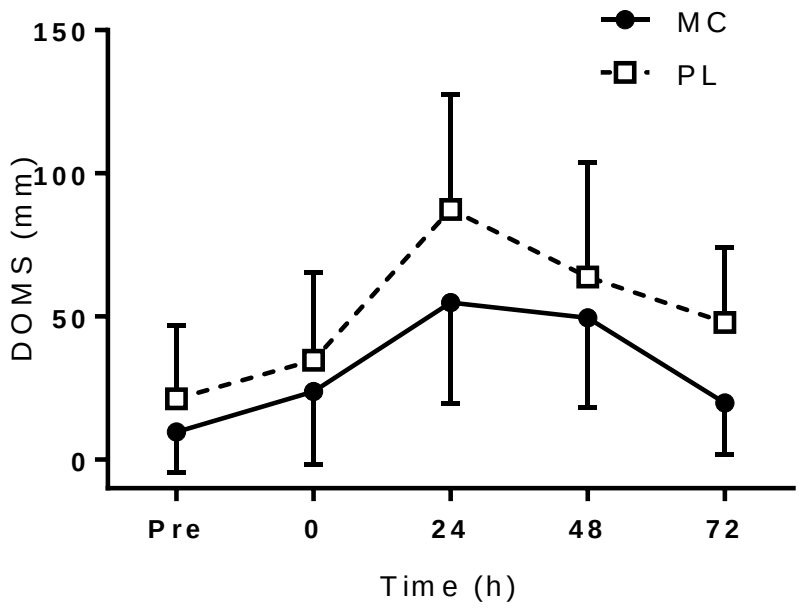

Figure 14. Muscle soreness (DOMS) post exercise-induced muscle damage in the Montmorency cherry (MC) (n = 10) and placebo (PL) (n = 10) groups. Values presented as mean ± SD.

Pain pressure threshold values pre-exercise at the rectus femoris (RF), vastus lateralis (VL) and medial head of the gastrocnemius (GM) were 48.1 ± 8.8 vs 35.2 ± 12.4 N (p = 0.014), 42.6 ± 9.8 vs 30.2 ± 10.1 N (p = 0.012), and 41.4 ± 11.2 vs 27.3 ± 12.1 N (p = 0.017), in the MC and PL groups, respectively. Absolute values of PPT were expressed as percentage change relative to pre muscle damage values prior to analysis. There was a main effect of time for pain pressure threshold

(PPT) percentage change at the RF ($F_{2.7, 48.5}$ = 8.6, p < 0.001), VL ($F_{4, 72}$ = 8.3, p < 0.001) and GM ($F_{4, 72}$ = 15.7, p < 0.001). At all three locations, PPT percentage change reached lowest levels at 24 h and then increased throughout recovery. There were no group differences in VL ($F_{1, 18}$ = 0.4, p = 0.524) or GM ($F_{1, 18}$ = 0.4, p = 0.548), but a trend for higher PPT in the MC group at the RF was observed ($F_{1, 18}$ = 3.7, p = 0.071, d = 0.59) (Figure 15). There were no interaction effects at the RF ($F_{2.7, 48.5}$ = 1.2, p = 0.322), VL ($F_{4, 72}$ = 1.1, p = 0.349) and GM ($F_{4, 72}$ = 0.7, p = 0.613).

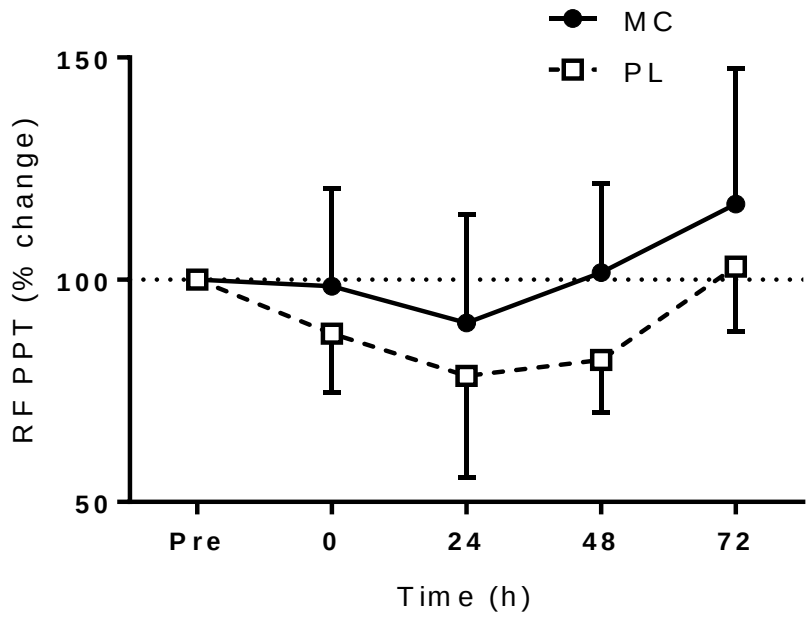

Figure 15. Percentage change from pre-exercise (pre) pain pressure threshold (PPT) at the rectus femoris (RF) post exercise-induced muscle damage in the Montmorency cherry (MC) (*n* = 10) and placebo (PL) (*n* = 10) groups. Values presented as mean ± SD.

5.3.2 Limb girth

Pre-exercise thigh girth was 50.1 ± 3.1 vs 50.6 ± 2.2 cm in the MC and PL groups (p = 0.675), respectively and pre-exercise calf girth was 36.1 ± 2.3 vs 36.2 ± 2.1 cm in the MC and PL groups, respectively (p = 0.902). Thigh and calf girths were unaffected post-exercise (time effects; $F_{2.4, 43}$ = 1.4, p = 0.256 and $F_{4, 72}$ = 0.2,

p = 0.946, for thigh and calf girths, respectively) and there were no differences between treatment groups ($F_{1, 18}$ = 0.7, p = 0.800 and $F_{1, 18}$ = 1.0, p = 0.342, for thigh and calf girths, respectively) or interaction effects ($F_{2.4, 43}$ = 0.4, p = 0.691 and $F_{4, 72}$ = 0.7, p = 0.572, for thigh and calf girths, respectively) (Table 11).

5.3.3 Hamstring stiffness and flexibility

Raw values for flexibility pre-exercise were 29.1 ± 5.4 vs 20.3 ± 9.0 cm in the MC and PL groups, respectively (15 cm being equivalent to touching toes) (p = 0.017). Flexibility was reduced for 48 h post-exercise but returned to baseline levels at 72 h in both groups ($F_{1.7, 30.5}$ = 4.6, p = 0.022). While the decrements appeared to be attenuated with MC, this was not significant ($F_{1, 18}$ = 2.2, p = 0.152) and there were no interaction effects ($F_{1.7, 30.5}$ = 0.8, p = 0.423) (Table 11).

5.3.4 Muscle function

Independent samples t tests determined that there were no significant group differences between absolute pre-exercise values of measures of muscle function except RSI (p = 0.595; p = 0.030; p = 0.951; and p = 0.758 for CMJ, RSI, MVC and 30 m sprint time, respectively). Absolute values of muscle function were expressed as percentage change relative to pre muscle damage values prior to analysis. All measures of muscle function were reduced post-exercise and progressively recovered throughout recovery (time effects; $F_{2.5, 45.5}$ = 11.7, p < 0.001; $F_{2.6, 47}$ = 4.5, p = 0.010; $F_{4, 72}$ = 8.5, p < 0.001; and $F_{2.4, 42.2}$ = 3.5, p = 0.033 for CMJ, RSI, MVC and 30 m sprint time, respectively). While recovery of these measures appeared to accelerate with MC, a group effect was only evident with CMJ ($F_{1, 18}$ = 7.0, p = 0.016, d = 0.66) (Figure 16). RSI ($F_{1, 18}$ = 0.4, p = 0.836), MVC ($F_{1, 18}$ = 0.001, p = 0.981) and 30 m sprint time ($F_{1, 18}$ = 0.7, p = 0.425) were not difference between treatments. There were no group x time interactions for CMJ ($F_{2.5, 45.5}$ = 1.4, p = 0.248), RSI ($F_{2.6, 47}$ = 1.2, p = 0.347), MVC ($F_{4, 72}$ = 0.9, p = 0.460), and 30 m sprint time ($F_{2.4, 42.2}$ = 0.6, p = 0.576).

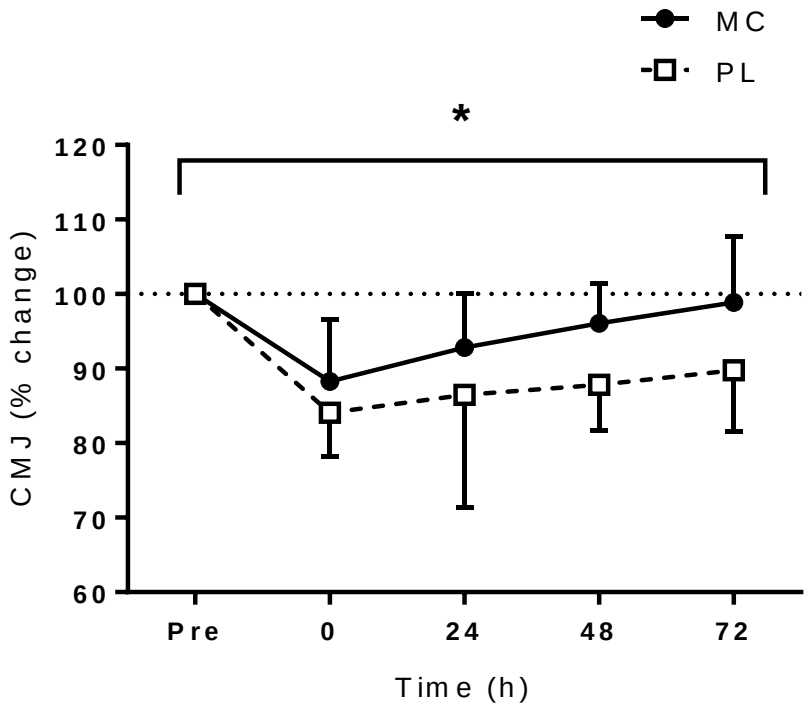

Figure 16. Percentage change from pre-exercise (pre) countermovement jump height (CMJ) post exercise-induced muscle damage in the Montmorency cherry (MC) (*n* = 10) and placebo (PL) (*n* = 10) groups. Values presented as mean ± SD. *denotes significantly higher CMJ in MC group. Significance at p < 0.05.

5.3.5 Blood indices

Independent samples *t* tests determined that there were no significant group differences between absolute pre-exercise values of CK and hsCRP (p = 0.122; p = 0.074, respectively) Paired samples *t* tests determined that total CK concentrations were not different between baseline and pre-exercise time-points for both MC (p = 0.091) and PL (p = 0.808) groups. Two-way group (2; MC vs PL) x time (5; pre, and 0, 24, 48 and 72 h post EIMD) repeated measures ANOVA demonstrated time effects for CK ($F_{1.9,\ 33.4}$ = 21.9, p < 0.001). Both groups experienced an increase in circulating CK which peaked 24 h post-exercise (421.8 ± 251.7 vs 579.0 ± 347.8 $IU \cdot L^{-1}$ in the MC and PL groups, respectively) and remained elevated for 72 h post-exercise (Figure 17) with no group ($F_{1,\ 18}$ = 1.7, p = 0.212) or interaction effects ($F_{1.9,\ 33.4}$ = 1.3, p = 0.285). Paired samples *t* tests determined that

circulating hsCRP were not different between baseline and pre-exercise time-points for both MC (p = 0.508) and PL (p = 0.064) groups. Two-way repeated measures ANOVA demonstrated that circulating hsCRP was unaffected by exercise in both treatment groups (time effect; $F_{1.6,\ 28.8}$ = 0.2, p = 0.764) and not different between groups (group effect; $F_{1,\ 18}$ = 0.08, p = 0.782 and interaction effect; $F_{1.6,\ 28.8}$ = 0.8, p = 0.450).

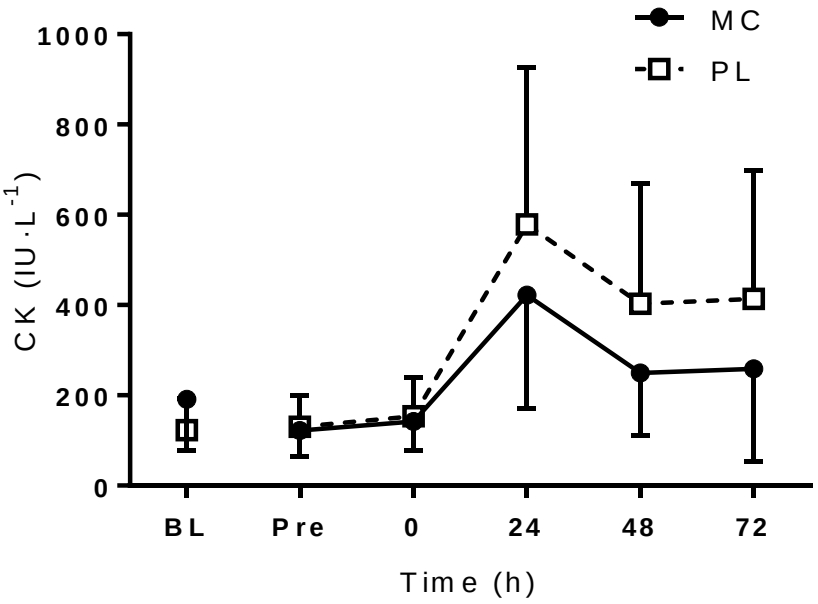

Figure 17. Total creatine kinase (CK) at baseline pre-supplementation (BL), before (Pre) and post exercise-induced muscle damage in the Montmorency cherry (MC) (*n* = 10) and placebo (PL) groups (*n* = 10). Values presented as mean ± SD.

Table 11. Values for dependent variables in response to muscle-damaging exercise, mean ± SD.

		Time post muscle-damaging exercise (h)					
Variable	**Group**	**BL**	**Pre**	**0**	**24**	**48**	**72**
DOMS, mm	MC	-	9.7 ± 14.2	23.8 ± 25.6	54.8 ± 35.3	49.6 ± 31.4	19.8 ± 18.2
	PL	-	21.3 ± 25.5	34.8 ± 30.4	87.4 ± 40.0	63.8 ± 39.9	47.9 ± 26.2
RF PPT, % (N)	MC	-	100 ± 0 (48.1 ± 8.8)	98.5 ± 21.9 (46.9 ± 11.6)	90.3 ± 24.5 (42.5 ± 10.8)	101.6 ± 20.1 (48.7 ± 12.9)	117.0 ± 30.6 (56.0 ± 17.5)
	PL	-	100 ± 0 (35.2 ± 12.4)	87.9 ± 13.3 (30.8 ± 10.6)	78.3 ± 22.8 (25.9 ± 6.9)	81.9 ± 11.8 (28.7 ± 10.0)	103.0 ± 14.5 (35.1 ± 8.8)
VL PPT, % (N)	MC	-	100 ± 0 (42.6 ± 9.8)	110.2 ± 29.8 (47.3 ± 18.0)	82.6 ± 28.4 (34.9 ± 13.6)	97.7 ± 22.6 (41.6 ± 12.6)	112.8 ± 43.7 (53.3 ± 23.3)
	PL	-	100 ± 0 (30.2 ± 10.1)	97.6 ± 14.1 (29.7 ± 11.8)	87.6 ± 21.4 (25.5 ± 7.2)	94.6 ± 16.9 (28.5 ± 10.2)	108.5 ± 17.2 (32.5 ± 10.5)
GM PPT, % (N)	MC	-	100 ± 0 (41.4 ± 11.2)	102.2 ± 22.8 (42.4 ± 15.5)	81.9 ± 29.1 (31.8 ± 8.8)	98.1 ± 20.7 (39.4 ± 9.4)	124.4 ± 25.9 (50.2 ± 12.2)
	PL	-	100 ± 0 (27.3 ± 12.1)	91.4 ± 15.7 (25.0 ± 12.1)	83.9 ± 24.7 (21.9 ± 8.7)	94.0 ± 23.9 (24.9 ± 10.2)	115.5 ± 21.7 (30.1 ± 9.9)
Thigh girth, % (cm)	MC	-	100 ± 0 (50.1 ± 3.1)	100.6 ± 1.0 (50.4 ± 3.0)	100.3 ± 1.0 (50.3 ± 3.0)	100.3 ± 0.9 (50.3 ± 3.0)	100.5 ± 1.0 (50.3 ± 2.8)
	PL	-	100 ± 0 (50.6 ± 2.2)	100.4 ± 1.5 (50.9 ± 2.6)	100.3 ± 0.8 (51.0 ± 2.4)	100.8 ± 1.3 (51.0 ± 2.5)	100.6 ± 1.6 (50.9 ± 2.4)
Calf girth, % (cm)	MC	-	100 ± 0 (36.1 ± 2.3)	100.1 ± 0.5 (36.2 ± 2.4)	100.0 ± 0.8 (36.1 ± 2.4)	100.1 ± 0.7 (36.1 ± 2.3)	100.3 ± 0.9 (36.2 ± 2.2)
	PL	-	100 ± 0 (36.2 ± 2.1)	99.8 ± 0.9 (36.1 ± 1.9)	99.9 ± 0.8 (36.2 ± 2.0)	99.9 ± 0.8 (36.2 ± 2.2)	99.8 ± 0.9 (36.2 ± 2.0)
Flexibility, % (cm)	MC	-	100 ± 0 (29.1 ± 5.4)	97.1 ± 11.0 (28.3 ± 6.4)	86.1 ± 19.9 (25.0 ± 7.2)	91.2 ± 27.2 (26.4 ± 9.3)	100.2 ± 20.2 (29.0 ± 7.8)
	PL	-	100 ± 0 (20.3 ± 9.0)	84.2 ± 13.3 (17.7 ± 9.0)	77.1 ± 23.1 (14.5 ± 6.1)	86.9 ± 17.4 (17.4 ± 8.3)	86.8 ± 16.5 (17.6 ± 9.0)

Table 11. Continued

		Time post muscle-damaging exercise (h)					
Variable	**Group**	**BL**	**Pre**	**0**	**24**	**48**	**72**
CMJ, % (cm)	MC	-	100 ± 0 (27.6 ± 2.6)	88.3 ± 8.3 (24.4 ± 3.8)	92.8 ± 7.3 (25.7 ± 3.8)	96.1 ± 5.3 (26.5 ± 3.0)	98.9 ± 8.8 (27.3 ± 4.2)
	PL	-	100 ± 0 (26.7 ± 4.5)	84.1 ± 5.8 (22.5 ± 4.5)	86.5 ± 15.0 (22.8 ± 4.4)	87.8 ± 6.1 (23.5 ± 4.6)	89.8 ± 8.3 (24.0 ± 5.0)
RSI, % (cm·s^{-1})	MC	-	100 ± 0 (102.8 ± 22.5)	86.5 ± 12.4 (88.6 ± 21.5)	94.1 ± 15.7 (97.2 ± 28.5)	96.6 ± 9.4 (99.0 ± 22.2)	103.9 ± 10.5 (107.0 ± 27.1)
	PL	-	100 ± 0 (81.5 ± 17.6)	93.5 ± 16.0 (74.7 ± 13.7)	91.1 ± 23.5 (72.0 ± 13.8)	92.0 ± 21.6 (73.0 ± 14.4)	99.2 ± 17.3 (80.2 ± 20.1)
MVC, % (N)	MC	-	100 ± 0 (394.3 ± 59.3)	87.1 ± 8.6 (347.1 ± 82.5)	90.9 ± 10.4 (362.7 ± 87.1)	95.7 ± 11.2 (381.3 ± 87.2)	95.0 ± 5.9 (376.5 ± 73.6)
	PL	-	100 ± 0 (392.2 ± 89.4)	91.1 ± 7.2 (354.1 ± 72.7)	91.6 ± 9.6 (355.4 ± 73.8)	93.5 ± 10.4 (361.8 ± 70.2)	92.3 ± 6.4 (375.9 ± 63.9)
30 m sprint time, % (s)	MC	-	100 ± 0 (5.32 ± 0.35)	102.0 ± 5.0 (5.42 ± 0.36)	101.4 ± 3.9 (5.39 ± 0.31)	102.7 ± 5.1 (5.45 ± 0.29)	101.0 ± 5.6 (5.37 ± 0.40)
	PL	-	100 ± 0 (5.28 ± 0.26)	103.6 ± 4.6 (5.46 ± 0.30)	102.0 ± 5.4 (5.37 ± 0.27)	106.1 ± 10.3 (5.58 ± 0.41)	103.2 ± 6.4 (5.43 ± 0.29)
CK, IU·L^{-1}	MC	191.1 ± 113.1	122.0 ± 58.5	144.9 ± 64.1	421.8 ± 251.7	260.6 ± 138.5	270.1 ± 204.8
	PL	123.1 ± 69.4	130.7 ± 68.4	154.0 ± 85.6	579.0 ± 347.8	403.1 ± 267.9	405.2 ± 284.0
hsCRP, mg·L^{-1}	MC	1.20 ± 0.98	1.63 ± 1.99	1.79 ± 1.87	2.15 ± 0.24	2.13 ± 1.97	1.56 ± 1.35
	PL	0.55 ± 0.35	1.81 ± 1.93	1.80 ± 1.87	1.73 ± 1.73	1.29 ± 1.14	1.71 ± 1.31

MC, Montmorency cherry group (n = 10); PL, placebo group (n = 10); BL, baseline pre-supplementation; %, % change from pre-exercise (Pre); DOMS, delayed onset muscle soreness; RF, rectus femoris; VL, vastus lateralis; GM, medial head of the gastrocnemius; PPT, pain pressure threshold; CMJ, countermovement jump; RSI, reactive strength index; MVC, maximal voluntary isometric contraction; CK, creatine kinase; hsCRP, high sensitivity C-reactive protein.

5.4 Discussion

This study sought to examine the efficacy of 8-day MC supplementation on recovery from muscle-damaging exercise in female dancers. This is the first study to identify the effects of MC in female participants following repeated-sprint exercise. It was hypothesised that MC consumption would reduce markers of muscle damage and inflammation in response to repeated-sprint activity. The data demonstrate that MC supplementation accelerated the recovery of CMJ and was associated with trends of reduced muscle soreness.

Immediately post-exercise, there was a decline in CMJ of 11.7 ± 8.3 and 13.5 ± 8.7% in MC and PL groups, respectively, and this was not different between groups (independent samples *t* test; $p = 0.205$). However, while CMJ remained below 90% of pre-exercise levels in the PL group, there was a clear acceleration in recovery in the MC group, which achieved 98.9 ± 8.8% of pre-exercise levels by 72 h post muscle-damaging exercise. This supports a recent study demonstrating an improvement in CMJ (Bell et al., 2016), and a number of studies demonstrating an accelerated recovery in other measures of muscle function with MC consumption (Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010). Previously, these improvements have been suggested to be attributable to a protective effect provided by a preload of MC; demonstrated by increased anti-oxidative status (TAS) (Howatson et al., 2010), lower oxidative stress (LOOH) (Bell, Walshe, et al., 2014), and reduced inflammation (hsCRP) prior to exercise and a preservation of muscle function immediately post exercise and throughout trial periods (Bell et al., 2015). In contrast, we reported no tangible differences in hsCRP with a preload of MC prior to exercise and both groups experienced a comparable decline in CMJ immediately post-exercise; indicating a similar degree of muscle damage.

It is important to note that some studies demonstrating differences between markers of inflammation and oxidative stress with an MC preload have required participants to consume a low polyphenolic diet throughout trial periods (Bell, Walshe, et al., 2014; Bell et al., 2015). The reported differences between PL and MC groups in these studies may therefore be explained by a reduced antioxidant capacity in the PL

group rather than an improvement elicited with MC supplementation, thereby limiting the generalisability of these results. Certainly, habitual dietary intakes of foods containing polyphenols contribute to typical anti-inflammatory and anti-oxidative status (Bonaccio et al., 2016; Scoditti, Capurso, Capurso, & Massaro, 2014). A notable strength of the current study was that participants were not restricted in their consumption of polyphenolic rich foods and were instructed to consume their habitual diets. This might explain the lack of difference between groups in levels of hsCRP prior to exercise. Given that there were no differences between groups in energy intake, macronutrient intake, and portions of polyphenolic foods throughout the study, it appears that attenuated inflammation with an MC preload is unlikely in true sporting scenarios, at least in the doses used in the present study. However, while concentrations of hsCRP were not different between baseline and pre-exercise, it is possible that other markers of inflammation, oxidative stress, and antioxidant capacity could have been improved with a preload of MC. For instance, Howatson et al. (2010) observed elevations in TAS before muscle-damaging activity with supplementation of MC compared to PL, despite all participants consuming typical dietary intakes with limited restriction. Moreover, to date, all studies investigating the efficacy of MC ingestion on EIMD and recovery have employed supplementation strategies involving both pre- and post-exercise consumption (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010; Kuehl et al., 2010; Levers et al., 2015). It is therefore difficult to identify the independent effects of a preload and of ingestion during recovery in isolation. Nonetheless, our findings suggest that it is not the initial muscle damage insult which is attenuated with MC, but rather it is likely that the secondary damage response (characterised as an increase in oxidative stress and/or inflammation; exacerbating the initial damage) is dampened.

The improvement of CMJ with MC may be explained by a protection against oxidative injury to the type II fibres recruited for such activity. Eccentric exercise is thought to selectively damage type II muscle fibres (Friden, Sjostrom, & Ekblom, 1983; Jones, Newham, Round, & Tolfree, 1986; Macaluso, Isaacs, & Myburgh, 2012) and this has implications on the force-generating capacity and velocity of shortening of the muscle during functional tasks (Byrne et al., 2004). Interestingly, evidence suggests that mitochondrial ROS production and/or release is potentiated

in type II fibres (Anderson & Neufer, 2006) and the activity of principle antioxidant enzymes including superoxide dismutase (SOD) (Criswell et al., 1993; Powers et al., 1994) and glutathione peroxidase (GPX) (Lawler et al., 1994; Powers et al., 1994) are lowest in type II fibres compared with type I fibres in rodent models. During periods of increased oxidant production (for instance intense exercise), both enzymatic (such as SOD and GPX) and non-enzymatic antioxidants collectively protect muscle fibres from oxidative injury (Powers & Jackson, 2008). Therefore, one could speculate that supplementation of non-enzymatic antioxidants may have contributed to the free radical scavenging capacity of type II fibres and preserve their muscle functionality during high power activities. Indeed, muscle force production has been increased in a rodent model by NO synthase inhibitors and NO scavengers (Kobzik, Reid, Bredt, & Stamler, 1994). However, while CMJ was improved with MC, no other measures of functional performance were different between groups. Given that the functional measures in the present investigation all require type II fibre recruitment, intuitively, we would have expected the measures to be equally affected by MC supplementation. Indeed, the lack of an accelerated recovery of MVC with MC is in contrast to a number of previous studies (Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010). Previously, the learning effect associated with muscle function measures and their novelty to the study sample has been put forward as a potential explanation for the lack of group differences. However, in the present investigation, we would expect the female dancers to be familiar with all performance variables given that dance is characterised by jumps (Paschalis et al., 2012), explosive movements (Westblad et al., 1995), and (though perhaps least familiar) sprint activity (Cohen et al., 1982). Certainly, the role of MC in accelerating the recovery of muscle function post EIMD remains unclear.

Subjective muscle soreness appeared to be lower prior to exercise following preload supplementation with MC (9.7 ± 14.2 mm) compared to PL (21.3 ± 25.5 mm); however, this was not a significant difference (independent samples *t* test; $p = 0.225$) and is likely explained by individual variation in such subjective measurements. However, supplementation with MC resulted in a trend for reduced subjective muscle soreness as well as increased PPT at the RF; which could have played a role in the observed improvements in CMJ. This is in line with a number of

investigations reporting reduced soreness and pain with MC supplementation (Bell et al., 2016; Connolly et al., 2006; Kuehl et al., 2010; Levers et al., 2015). While muscle pain assessed with a VAS has been reduced with MC following 10 sets of 10 single-leg knee extensions at 80% 1-RM, in accordance with our findings, muscle tenderness measured using algometry only tended to be lower (Connolly et al., 2006). Recently, in a similar study, muscle soreness measured via algometry was reduced following 10 sets of 10 barbell back squats at 70% of 1-RM (Levers et al., 2015). Kuehl and colleagues (Kuehl et al., 2010) have also reported significantly smaller increases in feelings of muscular pain with MC following long distance running. In contrast, while none have demonstrated a negative effect of MC supplementation on DOMS, others have found no benefit (Bell et al., 2015; Bowtell et al., 2011; Howatson et al., 2010). In addition, the reductions in muscle soreness and pain previously reported have not always been accompanied with improvements in muscle function, and vice versa (Bell et al., 2015; Bowtell et al., 2011; Levers et al., 2015). The inconsistencies in the literature could be explained by the disparities in exercise protocol employed. Indeed, muscle soreness has been associated with increases in inflammation following exercise (Kraemer et al., 2004). In the current investigation, hsCRP was not different between groups across all trial periods, and limb girth (an indirect measure of inflammation, swelling and oedema (Smith, 1991; van Someren et al., 2005)) was unaffected by the exercise. Compared to marathon running (Howatson et al., 2010) and high intensity cycling exercise (Bell et al., 2015), where CRP has been shown to increase 24 and 48 h post-exercise, the SP is a less metabolically challenging exercise and likely unable to induce a large inflammatory response. Indeed, the use of the LIST has previously been shown to result in no significant changes in CRP (Bailey, Williams, Betts, Thompson, & Hurst, 2011; Leeder et al., 2014). The reductions in inflammation previously associated with MC supplementation may well only be detected following exercise with a high metabolic component compared to more conventional activity. It is conceivable that the exercise stimulus in this study was insufficient to affect systemic inflammation and therefore insufficient to detect larger magnitudes of change in muscle soreness and PPT with MC consumption.

While total CK appeared to be reduced with MC ingestion, this did not reach significance and previous studies have reported similar findings. Bowtell et al.

(2011) reported that CK tended to be lower in a MC trial compared to placebo (p = 0.055), and a study in horses also observed trends in reduced CK following muscle-damaging treadmill exercise in favour of MC (p = 0.054) (Ducharme et al., 2009). Yet, aside from these trends, a reduction in CK following muscle-damaging exercise has not been reported with MC in previous investigations (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Howatson et al., 2010; Levers et al., 2015). The lack of significant differences between groups is perhaps unsurprising given the CK response shows high levels of inter-individual variability which has been attributed to factors including training status and supposed high and low responders (Brancaccio et al., 2007); with genetic variations in the coding of myofibrillar proteins influencing the phenotypic response to muscle-damaging exercise (Baird et al., 2012; Clarkson et al., 2005). The ability to demonstrate differences is also affected by the lower CK values elicited by the exercise protocol which is less than other damaging protocols (for instance following marathon running (Howatson et al., 2010), and an adapted LIST protocol (Bell et al., 2016); where CK levels peaked at > 2000 and > 1000 $IU \cdot L^{-1}$, respectively at 24 h post-exercise). In addition, females have lower resting CK than males (Fu, You, & Kong, 2002) and generally have an attenuated CK response after exercise (Amelink, Kamp, & Bar, 1988; Stupka et al., 2000), owing in part to the antioxidant properties of 17β-oestradiol (Bar, Amelink, Oldenburg, & Blankenstein, 1988; Tang, Abplanalp, Ayres, & Subbiah, 1996).

The data which demonstrate no group differences in hsCRP and CK do not wholly support the literature which traditionally suggests attenuated symptoms of EIMD with MC is attributable to reduced muscle damage and inflammation; at least following repeated-sprint exercise in females. However, there are limitations associated with these systemic markers, not least because they are surrogate and indirect indices of muscle damage and inflammation. The current study was also limited by a lack of measurement of oxidative stress and antioxidant capacity. Though some investigations have failed to identify differences in some oxidative stress and antioxidant status markers, increases in TAS and reductions in uric acid and TBARS (Howatson et al., 2010), and reductions in levels of protein carbonyls (Bowtell et al., 2011) and LOOH (Bell, Walshe, et al., 2014) have been demonstrated following strenuous exercise with supplementation of MC compared

to placebo. It is conceivable that enhanced antioxidant status and redox balance may have contributed to the improvements in CMJ and trends in reduced muscle soreness observed with MC in this study. Future research should include measurement of a variety of systemic indices associated with muscle damage, inflammation and oxidative stress to provide greater insight into specific mechanisms influencing improved muscle function and pain with MC ingestion. Specifically, recent evidence suggests a reduction in muscle catabolism (creatinine, total protein and bilirubin), physiological stress (cortisol, testosterone, AST and ALT), and an increase in the immune cell response (lymphocytes and white blood cells) with 10-day supplementation of powdered MC capsules surrounding an acute bout of resistance exercise (Levers et al., 2015). While these provide potential mechanistic links for the beneficial effects offered by MC, much of the findings in the study were determined by *post-hoc* pairwise comparisons, often without significant main effects. The authors' approach to statistical analysis brings into question the credibility and strength of the conclusions drawn and further research is required to substantiate these initial findings.

It has been suggested that repeated MC supplementation offers cumulative effects, which might be responsible for reducing the negative symptoms associated with EIMD (Bell et al., 2015). This might explain the accelerated recovery in CMJ in the days following EIMD. Emerging evidence suggests that anthocyanins and other bioactive compounds have the potential to be stored. For instance, multiple doses of quercetin (a flavonoid metabolite which has a half-life of 11 to 28 h (Graefe et al., 2001; Hollman et al., 1997)) might result in plasma accumulation (Manach et al., 2004; Manach et al., 2005). The persistent presence of anthocyanin metabolites in human excreta post consumption of anthocyanin rich supplements (for instance in 24 h urine samples (Felgines et al., 2003) and 48 h faecal samples (Czank et al., 2013)) has also been suggested to indicate minor tissue accumulation (Kay et al., 2004). More recently, a study has demonstrated that 3-week supplementation of MC increased concentrations of a number of phenolic compounds in various tissues in a rodent model (Kirakosyan et al., 2015). However, it is yet to be determined whether this is also the case in humans, and in muscle tissue where anti-inflammatory and anti-oxidative activities would arguably be of greatest use following EIMD. In addition, it is possible that a much longer duration and perhaps more frequent MC

supplementation strategy than that employed in the present investigation is required to maintain high levels of bioactive compounds required to promote accumulation and have the potential to influence recovery processes *in vivo*. Having said this, while tissue accumulation of polyphenolic compounds was not investigated, similar supplementation strategies have demonstrated favourable effects of MC on muscle damage and recovery (Bell, Walshe, et al., 2014; Bell et al., 2015; Connolly et al., 2006; Howatson et al., 2010).

5.5 Perspectives

This chapter addressed the third aim of the thesis: *'to investigate the influence of Montmorency tart cherry juice supplementation on exercise-induced muscle damage in female dancers'*. The results from this study resulted in the rejection of the null hypothesis, concluding that Montmorency tart cherry juice (MC) supplementation had a significant influence on exercise-induced muscle damage in female dancers. However, it should be noted that only one dependent variable was significantly improved with MC. This investigation examined the effect of MC on exercise recovery in females using robust measures of muscle function, muscle soreness, muscle damage, and inflammation in a controlled laboratory environment. In addition, limitations associated with previous research were addressed as the efficacy of MC for recovery was examined following an exercise protocol and in a population which has not been previously investigated. The main findings of this study were that 8-day MC supplementation improved recovery of muscle function (CMJ) and tended to lower muscle soreness compared to PL. No other markers were favourably affected by MC consumption. While some measures were not affected, these benefits are nonetheless an important consequence of MC supplementation and importantly have been observed following activity which is considered less damaging yet more conventional than those previously investigated. However, the external validity of these results is limited to similar populations and the exercise stimulus employed, and cannot be generalised to wider groups (please refer to section 7.3). Given that the muscle damage response is dependent upon exercise mode (Proske & Morgan, 2001) and training status (McHugh, 2003), future research should consider the effect of MC supplementation in dancers of different levels and

stages of their professional careers, and specifically following different styles of dance. Nonetheless, this research adds to the existing body of knowledge from previous research from our own laboratory and data from others. It also provides new information for the novel application of MC to wider groups. In particular, to females who would benefit from a practical nutritional intervention to help attenuate the symptoms of muscle damage and improve recovery on subsequent days. Indeed, in dance populations, optimal recovery and maintaining an ability to perform on a daily basis is often the primary goal. Moreover, antioxidant rich nutritional interventions are likely to contribute to the maintenance of immune function, and the prevention of illness. Finally, cherries are considered a nutrient dense food, with significant amounts of bioactive food components, with a relatively low caloric content (McCune et al., 2011). As a result, MC supplementation may be a practical intervention to help reduce some symptoms of muscle damage in female dancers, whilst also providing a relatively low caloric supplement to contribute to improving energy balance.

6 Whey protein hydrolysate supplementation and exercise-induced muscle damage in female dancers

6.1 Introduction

Exercise has been shown to increase protein turnover and amino acid oxidation (Evans, 1991) and this might be exacerbated in EIMD paradigms given the structural damage to skeletal muscle that might occur. Indeed, rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) are increased following unaccustomed, muscle-damaging exercise, and while this has been suggested to be unrelated to muscle contraction performed (Phillips et al., 1997), others suggest that MPS appears to be greater following eccentric compared to concentric contractions (Eliasson et al., 2006; Moore et al., 2005); perhaps mediated through a combination of greater tension and stretching of the muscle (Eliasson et al., 2006). However, at least in the fasted state (and although the associated rise in insulin with exercise diminishes the catabolism of protein (Beelen et al., 2010; Tipton, 2008)) there is a negative net muscle protein balance which does not become positive post-exercise if not compensated for through protein availability (Kumar et al., 2009; Phillips et al., 1997; Pitkanen et al., 2003). Consequently, protein intake might provide the required amino acids necessary for improving protein balance, which is crucial for repairing damaged structural proteins (Saunders, 2007; Tipton, 2008), and thus attenuating the negative symptoms associated with muscle damage.

A number of different forms of protein and their analogues have been investigated for their efficacy in ameliorating muscle damage and recovery; including (among others) whey (Burnley, Olson, Sharp, Baier, & Alekel, 2010; Roberts et al., 2011), casein (Flakoll, Judy, Flinn, Carr, & Flinn, 2004; Saunders et al., 2009), branched-chain amino acids (BCAA) (Areces et al., 2014; Howatson, Hoad, et al., 2012; Jackman et al., 2010), β-hydroxy β-methylbutyrate (Gonzalez et al., 2014; Nunan, Howatson, & van Someren, 2010; van Someren et al., 2005; Wilson, Fitschen, et al., 2013), milk-based products (Cockburn et al., 2013; Rankin, Stevenson, & Cockburn, 2015), and plant-based proteins (Coutinho, Cerqueira, Rodrigues, Porto, & Pierucci, 2014; Kalafati et al., 2010). Of contemporary interest is supplementation with hydrolysed proteins. These supplements are pre-digested proteins that are partially broken-down when exposed to heat, enzymes, or acids; producing large quantities of shorter peptides chains. As such, it is recognised that protein hydrolysates are more readily digested and absorbed, and increase

circulating amino acid concentrations more rapidly than intact proteins (Koopman et al., 2009; Manninen, 2004; Morifuji et al., 2010; Silk et al., 1979). Recently, the efficacy of whey protein hydrolysate (WPH) supplementation on reducing markers of muscle damage and accelerating recovery has received attention in the literature. The evidence for WPH in combination with carbohydrate are encouraging; with reported decreases in systemic indices of muscle damage (Hansen et al., 2015; Lollo et al., 2014), increases in satellite cell proliferation (Farup et al., 2014), alterations in signalling associated with muscle protein turnover (Rahbek et al., 2015), and accelerated physical (Cooke et al., 2010; Hansen et al., 2015) and psychological (Hansen et al., 2015) recovery. Data also appear to suggest that when consumed in isolation, there is greater benefit of WPH over other forms of whey for EIMD with both acute (Buckley et al., 2010) and more long-term (Lollo et al., 2014) supplementation strategies.

Preliminary data regarding WPH supplementation are promising, particularly as improvements have been demonstrated in recreationally active (Farup et al., 2014; Rahbek et al., 2015) and highly trained individuals (Hansen et al., 2015; Lollo et al., 2014). However, presently, the efficacy of WPH in accelerating recovery from EIMD has been investigated following acute eccentric/resistance exercise bouts (Buckley et al., 2010; Cooke et al., 2010; Farup et al., 2014; Rahbek et al., 2015) or longer-term training programmes (Hansen et al., 2015; Lollo et al., 2014), and no study has examined effects following an acute bout of repeated-sprint exercise. Many other exercise paradigms and exercising populations would benefit from potential strategies to reduce EIMD and therefore warrant investigation. For instance, dance activity has been shown to elicit muscle damage (Rodrigues-Krause et al., 2014) previously. Certainly, this course of investigation lends support to this previous work and has demonstrated that dancers are susceptible to the symptoms associated with EIMD, which have implications on subsequent performance potential. Moreover, all investigations exploring the influence of WPH on EIMD and recovery have been conducted with male or mixed sex groups. Although there have been no reported sex differences in the basal and post-exercise rates of MPS and MPB (Fujita, Rasmussen, Bell, Cadenas, & Volpi, 2007; Miller et al., 2006), the literature examining the differences in the susceptibility to EIMD between men and women is largely equivocal (Dannecker et al., 2012; Enns & Tiidus, 2010).

Therefore, efficacy of WPH for attenuating muscle damage and accelerating recovery requires further research in female populations.

Therefore, the aim of this investigation was to examine the efficacy of WPH gel supplementation on physiological and functional recovery following a bout of exercise designed to cause temporary muscle damage in female dancers. This investigation aimed to provide new data on the application of WPH following an applied exercise protocol and in an understudied population. It was hypothesised that indices of EIMD would be attenuated by the consumption of the WPH gel. As such, this chapter sought to address the fourth aim of the thesis: *'to investigate the influence of whey protein hydrolysate supplementation on exercise-induced muscle damage in female dancers.'*

6.2 Materials and methods

6.2.1 Participants

6.2.1.1 Recruitment

Please refer to section 4.2.1.1 for details of recruitment strategy.

6.2.1.2 Sample size

Raw data pertaining to isometric strength could not be determined from previous WPH literature. As a result, the sample size was based on previous research demonstrating positive effects on isometric strength with other protein supplementation (Blacker, Williams, Fallowfield, Bilzon, & Willems, 2010; Etheridge, Philp, & Watt, 2008; Howatson, Hoad, et al., 2012); which recruited ≤ 10 participants per group. As a number of studies demonstrating positive effects for WPH have recruited sample sizes of < 10 per group (Cooke et al., 2010; Hansen et al., 2015; Lollo et al., 2014), this was deemed appropriate.

6.2.1.3 Participant characteristics

Twenty healthy female recreational dancers (mean ± SD age 20 ± 1 y; stature 165.9 ± 5.6 cm; body mass 61.8 ± 7.9 kg; and BMI 22.4 ± 2.8 kg·m^{-2}, respectively) from a university dance team volunteered to participate and provided written informed consent (Appendix A; all characteristics displayed in Table 12). Dietary intake was controlled for 24 h prior to exercise and for the duration of the data collection period (please refer to section 6.2.4). The study was conducted according to the guidelines of the Declaration of Helsinki and the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria (HLSMB041215) approved the experimental procedures.

Table 12. Participant characteristics, mean ± SD.

	WPH (*n* = 10)	**CHO (*n* = 10)**	***p* value[1]**
Characteristics			
Age (y)	20 ± 1	20 ± 1	0.331
Body mass (kg)	64.9 ± 7.5	58.7 ± 7.3	0.080
Stature (cm)	167.3 ± 5.2	164.5 ± 5.9	0.278
BMI (kg·m^{-2})	23.2 ± 3.4	21.6 ± 1.9	0.204
Dance training (y)	15 ± 5	11 ± 4	0.060
Dance training (h·week^{-1})	5.8 ± 2.6	5.2 ± 1.9	0.563
Total exercise (h·week^{-1})	9.2 ± 4.5	6.4 ± 2.5	0.101

[1]Whey protein hydrolysate (WPH) vs carbohydrate (CHO) compared by independent samples *t* test.

6.2.1.4 Dietary and exercise restrictions

For 24 h prior to, and for each of the testing days, participants were asked to avoid strenuous exercise, alcohol, caffeine, nutritional supplements (including vitamin and mineral tablets), and any anti-inflammatory drugs or alternative treatments (including massage and cold water immersion). For more details please refer to section 4.2.1.4. These restrictions were employed to limit the influence of diet and

physical activity on the dependent variables and ensured that observed effects were likely to be in response to the supplementation implemented within the study.

6.2.2 Pre-testing procedures

Participants were required to complete a menstrual cycle questionnaire (Appendix C) in order to determine the history and phase of the menstrual cycle. The questionnaire identified the contraceptive use of participants; eight were using an oral combination pill (all monophasic), six were using a progesterone only pill/implant/injection, and six were normally menstruating. All testing took place during the early/mid luteal phase or where applicable in the 14 days prior to a withdrawal bleed. Participants were initially required to attend the laboratory for familiarisation with the procedures and the exercise protocol was described. Finally, participants were required to complete three maximal voluntary isometric contractions (MVC) of the knee extensors (described in detail in section 4.2.5.3.3); the peak of which was used for stratified randomisation. For more details regarding pre-testing procedures, please refer to section 4.2.2. Following pre-testing procedures (~1-2 weeks prior to data collection), participants were then required to attend the laboratory on four further occasions, each following an overnight fast. Participants were tested at the same time on subsequent days (± 1 h) to account for diurnal variation.

6.2.3 Experimental protocol

This study adopted an independent groups design and used stratified randomisation (described in section 4.2.2) to assign participants to one of two groups; a whey protein hydrolysate supplementation group (WPH) or an isoenergetic carbohydrate supplementation group (CHO). The study was conducted in a double-blind manner. Following written informed consent and pre-testing procedures (described in section 4.2.2), participants attended the laboratory for a further four consecutive days. Participants were provided with standardised meals 24 h prior to initial testing and were fasted for ≥10 h except for water, which was consumed *ad libitum.* On arrival at the laboratory, participant characteristics (stature measured to the nearest 1 mm

(stretch stature technique, Model 220, Seca Ltd, Birmingham, UK) and body mass to the nearest 0.1 kg (Model 876, Seca Ltd, Birmingham, UK)) and baseline measures of dependent variables were recorded. On completion of these baseline measures participants completed the exercise protocol designed to induce muscle damage. After a 2 min rest following this exercise, participants consumed a dose of the WPH or CHO supplement within 10 min and the aforementioned baseline measures were repeated. Before leaving the laboratory, participants consumed a standardised breakfast meal and a supplement was provided to be consumed 2 h post-exercise. Baseline measures were then repeated following an overnight fast and in the same order for the following 3 days after the exercise; 24, 48, and 72 h post damaging exercise. During this time, all food was provided and participants were required to consume a supplement 30 - 60 min prior to subsequent morning visits and prior to their evening meal for the two days following the exercise, and the final supplement consumed prior to final measurements at 72 h post EIMD. Please refer to Figure 18 below for an illustration of the study design and the following sections for details regarding all data collection procedures. Protocols and measurement of dependent variables were completed in an indoor sporting facility and environmental conditions were controlled (temperature, 17.2 ± 0.3^0C; pressure, 1009.9 ± 12.1 hpa; humidity, 32.8 ± 5.0%).

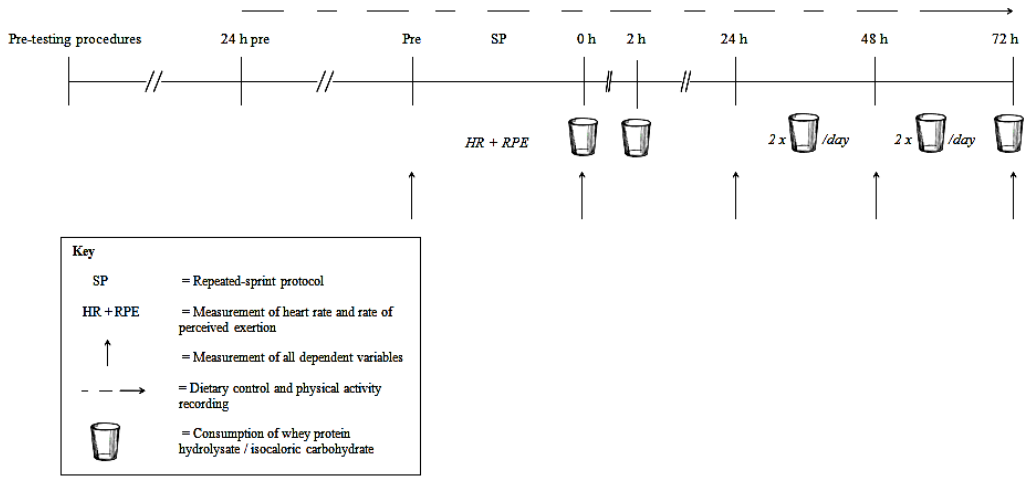

Figure 18. Schematic of testing protocol.

6.2.4 Dietary control

Food intake was controlled throughout all trial periods; breakfast, lunch, evening meals as well as regular snacks were provided (please refer to Table 13 for an example of the food provided each day).

Table 13. Standardised daily meal plan for participants over the four-day data collection period.

Meal	Food and drink provided
Breakfast	2 x white bread, toasted, with butter and strawberry jam
	1 x glass of milk
Lunch[1]	1 x sandwich or salad
	1 x packet of crisps
	1 x fruit smoothie
Evening Meal[1]	1 x curry or chilli
Snacks	1 x banana
	1 x cereal bar
	1 x packet of jelly sweets
	1 x yoghurt

[1]The meals did not deviate from this standardised plan, however specific foods and flavours provided during lunch and the evening meal were altered each day to ensure a varied diet and to avoid monotony.

Dietary control was applied to ensure that sufficient amounts of carbohydrate (5-7 $g \cdot kg^{-1} \cdot day^{-1}$) (Burke et al., 2006) and protein (1.2-1.7 $g \cdot kg^{-1} \cdot day^{-1}$) (Tipton & Wolfe, 2004) recommended for athletic populations were met by all participants (Table 14). In addition, particular care was taken to ensure that macronutrient requirements following muscle-damaging exercise were achieved. Participants consumed their assigned supplement within 10 min of EIMD and were provided with a standardised mixed macronutrient breakfast following testing each morning. Specifically, participants consumed 1.2 ± 0.1 and 1.7 ± 0.2 $g \cdot kg^{-1}$ of carbohydrate and 0.6 ± 0.1 and 0.3 ± 0.1 $g \cdot kg^{-1}$ of protein in the WPH and CHO groups

respectively within 45-60 min of exercise. Therefore in the early recovery period quantities of carbohydrate thought to saturate muscle glycogen resynthesis (1-1.2 g·kg^{-1}) and quantities of protein thought to support MPS (0.25-0.3 g·kg^{-1}) (Thomas, Erdman, & Burke, 2016) were consumed. The dietary control employed in this study was to ensure that any effect observed was not related to insufficient protein in the CHO group or insufficient carbohydrate in the WPH group. Consequently, it was anticipated that (given supplements were isocaloric and there were no differences in total daily energy intake) any group differences would be attributable to the additional protein provided in the WPH group. No changes in body mass were observed between the initial testing day (day 1) and the final testing day (day 4) in both treatment groups (paired samples *t* test; $p = 0.335$ and $p = 0.212$ in the WPH and CHO groups, respectively), demonstrating that participants were likely in energy balance.

Table 14. Daily dietary intake of participants over the four-day data collection period[1], mean ± SD.

		Excluding Supplements		Including Supplements	
Variable		**WPH**	**CHO**	**WPH**	**CHO**
Energy	kcal	2066 ± 108	2019 ± 183	2220 ± 108	2173 ± 183
	MJ	8.6 ± 0.5	8.4 ± 0.8	9.3 ± 0.5	9.1 ± 0.8
Carbohydrate	g·kg^{-1}	5.0 ± 0.7	5.5 ± 0.9	5.0 ± 0.7*	6.2 ± 1.0*
	%TEI	61 ± 3	63 ± 2	58 ± 3*	66 ± 2*
Protein	g·kg^{-1}	1.2 ± 0.2	1.3 ± 0.2	1.8 ± 0.2*	1.3 ± 0.2*
	%TEI	15 ± 1	15 ± 1	21 ± 1*	14 ± 1*
Fat	g·kg^{-1}	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.2
	%TEI	25 ± 3	24 ± 1	23 ± 3	23 ± 1

[1]As determined using dietary analysis software (Nutritics Ltd, Swords, Ireland). WPH, whey protein hydrolysate group ($n = 10$); CHO, carbohydrate group ($n = 10$); %TEI, percentage of total energy intake. *denotes significant difference between groups ($p < 0.05$).

6.2.5 Supplementation

Alongside all food intake described above (with no significant differences in energy and macronutrient intakes between groups), participants were provided with either WPH or isocaloric CHO supplementation in gel form to consume post EIMD and were instructed on ingestion frequency and timing. The daily dose was two bolus amounts of the WPH or CHO gel. On the day of muscle-damaging exercise, these doses were consumed immediately post the exercise protocol and 2 h post-exercise. For the following two days, these doses were consumed prior to breakfast (30 - 60 min prior to laboratory visits) and their evening meal, and a final supplement was consumed prior to final measurements at 72 h post-exercise. Each participant consumed seven supplements in total over the four-day period. This is based on recent work demonstrating an effect when WPH is consumed for three days following EIMD (Farup et al., 2014; Rahbek et al., 2015). Plasma essential amino acids have been typically reported to peak 30 - 60 min following WPH ingestion (Koopman et al., 2009; Morifuji et al., 2010; Power et al., 2009; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). In addition, the consumption of 20 g of protein (equivalent of 9 g essential amino acid) is thought to maximise MPS in the immediate post-exercise period; and that regular intakes may maintain MPS throughout the day (Beelen et al., 2010). Therefore, the present supplementation strategy (20 g protein of which 7 g BCAA, twice per day) allows for optimal plasma concentrations both coinciding with the laboratory visits and in the hours and days post-exercise when MPS is thought to persist (Phillips et al., 1997).

Both WPH and CHO gels were lemon flavoured, isovolumetric and isocaloric, and were microbiologically screened and Informed Sport tested. An isocaloric CHO supplement was chosen to exclude the potential effect of increased energy associated with added protein in recovery processes. Supplements were provided in identical packaging (Science in Sport Ltd, Farringdon, London) and subsequently labelled in a double-blind manner (by an individual not directly involved in the research). The provision of protein in the form of a gel is an innovative delivery system that has yet to be explored. This medium might be capable of delivering large amounts of protein in an arguably more practical and convenient way compared to traditional supplementation methods (ie. powder and shaker).

Participants were asked to keep supplements in a cool and dark place as per manufacturer instructions in order to minimise the potential degradation of bioactive compounds; particularly as whey proteins are relatively heat labile (Ismail & Gu, 2010). Following all data collection periods, only *n* = 3 participants correctly identified which supplement they had consumed. Please refer to Table 15 for information regarding nutritional composition of the supplements.

Table 15. Nutritional composition of the supplements per serving.

	WPH	**CHO**
Serving size (mL)	78	78
Energy (kcal)	88	88
Energy (kJ)	368	368
Protein (g)[1]	20	0
Carbohydrate (g)	1.8	21.8
Fat (g)	0.1	0.1

[1]According to manufactures' information, WPH contains 7 g BCAA. WPH, whey protein hydrolysate gel; CHO, carbohydrate gel.

6.2.6 Exercise protocol

Prior to baseline measurement of muscle function and prior to the exercise protocol, participants completed a standardised warm up. Please refer to section 4.2.4.1 for details. Participants completed the SP which briefly involves 15 x 30 m sprints separated by 60 s rest to induce muscle damage. Please refer to section 5.2.5 for details regarding this protocol and the rationale for its use. RPE and HR were also collected after each sprint effort; these are described in sections 5.2.5.1 and 5.2.5.2.

6.2.7 Dependent variables

The following dependent variables were measured pre, immediately post (0 h), and 24, 48, and 72 h post muscle-damaging exercise.

6.2.7.1 Muscle soreness

Muscle soreness was assessed subjectively using a VAS and objectively using algometry for PPT at three locations (rectus femoris (RF), vastus lateralis (VL), medial head of the gastrocnemius (GM)). Please refer to sections 4.2.5.1 and 5.2.6.1.2 for detailed information.

6.2.7.2 Limb girth

Thigh and calf girth were measured to assess inflammatory swelling and oedema. Please refer to section 4.2.5.2 for detailed information.

6.2.7.3 Hamstring stiffness and flexibility

Hamstring and lower back flexibility were measured using the sit and reach test. Please refer to section 5.2.6.3 for detailed information.

6.2.7.4 Muscle function

Muscle function was assessed via countermovement jump height (CMJ), reactive strength index (RSI), maximal voluntary isometric contraction of the knee extensors (MVC) and 30 m sprint time. Please refer to section 4.2.5.3 for detailed information.

6.2.7.5 Blood sampling and analysis

Blood samples (10 mL) were collected via venepuncture from the antecubital fossa area into serum gel vacutainers. After allowing samples to rest and clot at room temperature for a minimum of 20 min, samples were centrifuged for 15 min (4°C) at 3000 RCF in order to obtain serum. The aliquots were stored at -80°C for later analysis of total CK. Due to difficulties with blood sampling, data for a single time

point was missing out of a total of 100; the group mean was used to complete the data set. Serum total CK concentrations were determined spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, Burgess Hill, UK). Please refer to section 4.2.5.4.1 for detailed information.

6.2.8 Statistical analysis

Previously described statistical analysis methods were employed (please refer to section 4.2.6). Results are presented as means ± SD. For the purpose of data analysis, all dependent variables except for DOMS and CK are expressed as a percentage change relative to pre muscle damage values to account for inter-individual variability. Statistical software (IBM SPSS V22, IBM, Armonk, USA) was used for inferential analysis and statistical significance was accepted at the $p \leq 0.05$ level *a priori*. Two-way group (2; WPH vs CHO) x time (5; pre, and 0, 24, 48 and 72 h post EIMD) repeated measures analysis of variance (ANOVA) were performed for each dependent variable to assess for differences in group and time. Significant main effects were analysed using the Least Significant Difference test (LSD) for adjustment for multiple comparisons. Independent samples *t* tests were conducted on peak HR, peak RPE, fatigue, and total and mean sprint time to examine differences in exercise intensity during the SP between groups. Where appropriate, Cohen's D effect sizes (*d*) were calculated with the magnitude of effects considered small (0.2), medium (0.5) and large (> 0.8).

6.3 Results

All sampling distributions were considered normally distributed and there were no group differences in the absolute pre-exercise values of all dependent variables (independent samples *t* test, all $p > 0.05$). Time effects were observed for all dependent variables ($p < 0.05$) except limb girth, clearly showing evidence of EIMD. Independent samples *t* tests determined no differences between WPH and CHO groups for total sprint time (80.17 ± 5.43 vs 81.96 ± 3.26 s), mean sprint time (5.34 ± 0.36 vs 5.46 ± 0.22 s), fatigue (3.49 ± 1.69 vs 4.63 ± 1.69%), and peak HR (183 ± 14 vs 186 ± 21 bpm) and peak RPE (17 ± 3 vs 16 ± 2) during the SP, thereby

providing evidence that the exercise intensity was similar between groups. All dependent variable data are presented in Table 16.

6.3.1 Muscle soreness

Pre-exercise muscle soreness was 0.0 ± 0.0 vs 1.0 ± 2.5 mm in the WPH and CHO groups, respectively (p = 0.244). Delayed onset muscle soreness increased immediately post-exercise and remained elevated throughout recovery in both groups ($F_{4, 72}$ = 26.4, p < 0.001), peaking at 48 h post-exercise (56.7 ± 17.8 vs 71.2 ± 45.0 mm in the WPH and CHO groups, respectively). There were no group differences ($F_{1, 18}$ = 1.2, p = 0.298), or interaction effects ($F_{4, 72}$ = 1.3, p = 0.288) for DOMS (Figure 19). Pain pressure threshold (PPT) values pre-exercise at the rectus femoris (RF), vastus lateralis (VL) and medial head of the gastrocnemius (GM) were 61.1 ± 18.2 vs 52.6 ± 14.7 N (p = 0.270), 61.0 ± 17.5 vs 50.9 ± 15.6 N (p = 0.191), and 60.6 ± 20.4 vs 48.6 ± 17.8 N (p = 0.177), in the WPH and CHO groups, respectively. There was a main effect of time for PPT percentage change at the RF ($F_{2.4, 43.9}$ = 9.1, p < 0.001), VL ($F_{2.4, 42.8}$ = 8.1, p = 0.001) and GM ($F_{4, 72}$ = 8.8, p < 0.001). At all three locations, PPT percentage change reached lowest levels at 24 h and then increased throughout recovery. There were no group differences ($F_{1, 18}$ = 0.2, p = 0.662; $F_{1, 18}$ = 0.2, p = 0.660; and $F_{1, 18}$ = 0.3, p = 0.566, for RF, VL and GM, respectively) and no interaction effects ($F_{2.4, 43.9}$ = 0.2, p = 0.840; $F_{2.4, 42.8}$ = 0.5, p = 0.658; and $F_{4, 72}$ = 0.4, p = 0.827, for RF, VL and GM, respectively) for PPT.

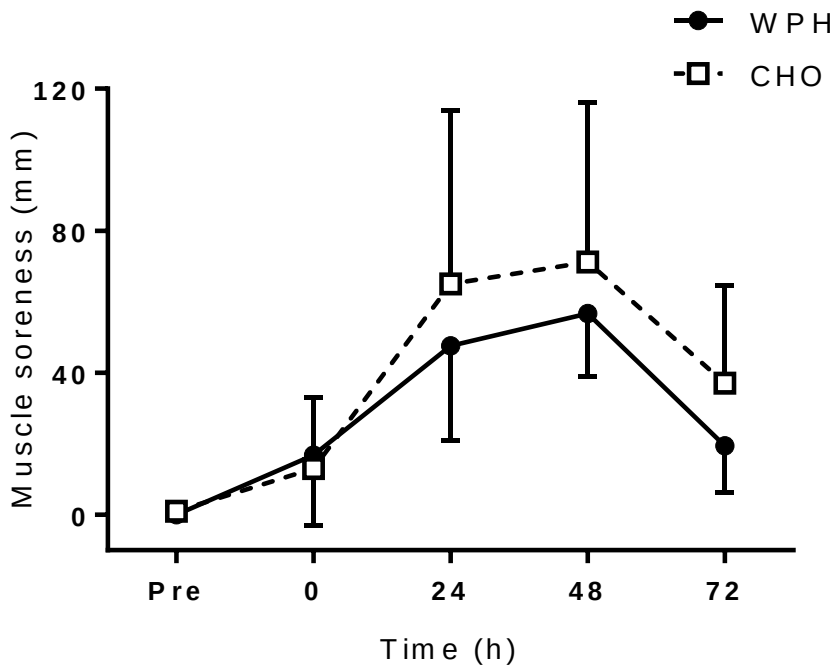

Figure 19. Muscle soreness (DOMS) post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (*n* = 10) and carbohydrate (CHO) (*n* = 10) groups. Values presented as mean ± SD.

6.3.2 Limb girth

Pre-exercise thigh girth was 51.9 ± 4.4 vs 48.9 ± 3.5 cm in the WPH and CHO groups, respectively ($p = 0.086$), and pre-exercise calf girth was 36.8 ± 1.8 vs 35.0 ± 2.8 cm in the WPH and CHO groups, respectively ($p = 0.103$). Thigh and calf girths (Table 15) were unaffected post-exercise (time effects; $F_{1.9, 35.0} = 0.5$, $p = 0.598$ and $F_{4, 72} = 1.7$, $p = 0.152$, for thigh and calf girths, respectively) and there were no group differences ($F_{1, 18} = 0.3$, $p = 0.594$ and $F_{1, 18} = 0.1$, $p = 0.794$, for thigh and calf girths, respectively) or interaction effects ($F_{1.9, 35.0} = 1.2$, $p = 0.323$ and $F_{4, 72} = 0.6$, $p = 0.635$, for thigh and calf girths, respectively).

6.3.3 Hamstring stiffness and flexibility

Raw values for flexibility pre-exercise were 28.1 ± 5.8 vs 24.8 ± 7.6 cm in the WPH and CHO groups, respectively (15 cm being equivalent to touching toes) ($p = 0.291$). Flexibility was reduced throughout recovery ($F_{2.5, 45.7} = 11.5$, $p < 0.001$), with lowest levels observed at 48 h post-exercise in both groups (Figure 20). There was no main effect of group ($F_{1, 18} = 2.9$, $p = 0.104$). However, there was an interaction effect ($F_{2.5, 45.7} = 3.0$, $p = 0.050$), where flexibility was improved beyond baseline measures at 72 h in the WPH group, but had failed to recover in the CHO group ($p = 0.011$, $d = 1.3$).

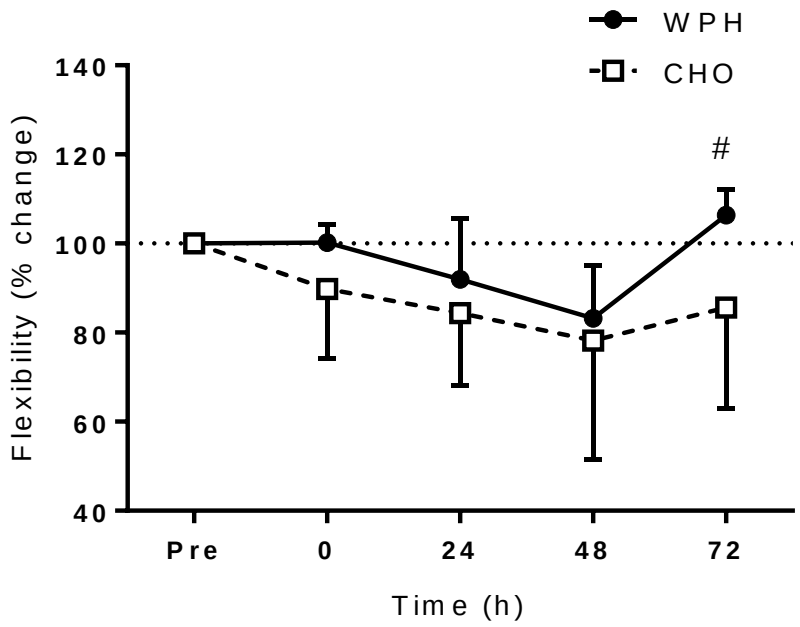

Figure 20. Hamstring stiffness and flexibility measured using the sit and reach test post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (*n* = 10) and carbohydrate (CHO) (*n* = 10) groups. Values presented as mean ± SD. #denotes significantly higher at 72 h in WPH group. Significance at p < 0.05.

6.3.4 Muscle function

Independent samples *t* tests determined that there were no significant group differences between absolute pre-exercise values of measures of muscle function ($p = 0.151$; $p = 0.066$; $p = 0.161$; and $p = 0.720$ for CMJ, RSI, MVC and 30 m sprint time, respectively). All measures of muscle function were reduced post-exercise and progressively recovered throughout recovery (time effects; $F_{4, 72} = 14.0$, $p < 0.001$; $F_{4, 72} = 12.7$, $p < 0.001$; $F_{4, 72} = 15.0$, $p < 0.001$; and $F_{4, 72} = 3.3$, $p = 0.016$ for CMJ, RSI, MVC and 30 m sprint time, respectively). While recovery of these measures appeared to accelerate with WPH, a group effect was only evident with RSI ($F_{1, 18} = 6.99$, $p = 0.016$, $d = 0.6$) (Figure 21). CMJ ($F_{1, 18} = 0.5$, $p = 0.490$), MVC ($F_{1, 18} = 1.2$, $p = 0.287$) and 30 m sprint time ($F_{1, 18} = 0.1$, $p = 0.860$) were not different between treatments. There were no group x time interactions for CMJ ($F_{4, 72} = 1.5$, $p = 0.212$), RSI ($F_{4, 72} = 1.7$, $p = 0.170$), MVC ($F_{4, 72} = 1.3$, $p = 0.282$), and 30 m sprint time ($F_{4, 72} = 0.4$, $p = 0.796$).

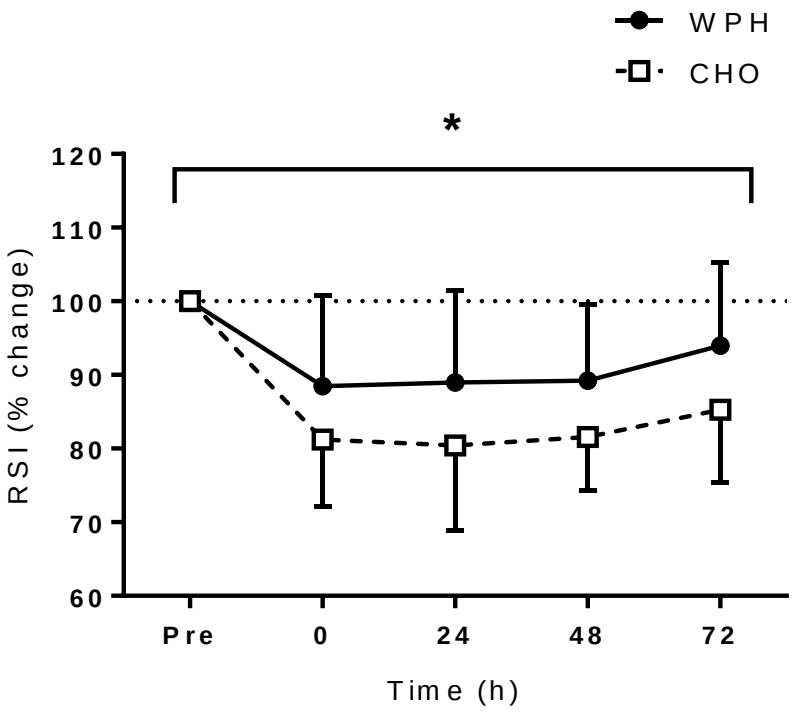

Figure 21. Reactive strength index (RSI) post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (*n* = 10) and carbohydrate (CHO) (*n* = 10) groups. Values presented as mean ± SD. *denotes significantly higher RSI in WPH group. Significance at p < 0.05.

6.3.5 Creatine kinase

Pre-exercise concentrations of CK were 157.9 ± 120.5 vs 101.6 ± 36.5 IU·L^{-1} in the WPH and CHO groups, respectively (p = 0.175). Both groups experienced an increase in circulating total CK ($F_{2.0, 35.4}$ = 19.1, p < 0.001), which peaked 24 h post-exercise (257.6 ± 103.6 vs 332.6 ± 155.9 IU·L^{-1} in the WPH and CHO groups, respectively) and remained elevated throughout recovery. There were no main effects of group ($F_{1, 18}$ = 0.7, p = 0.408). However, there was an interaction effect ($F_{2.0, 35.4}$ = 7.4, p = 0.002) and reductions in CK were greater following WPH consumption at 48 h compared to CHO (p = 0.031, ES= -1.1); where CK remained elevated throughout the 72 h recovery period (Figure 22).

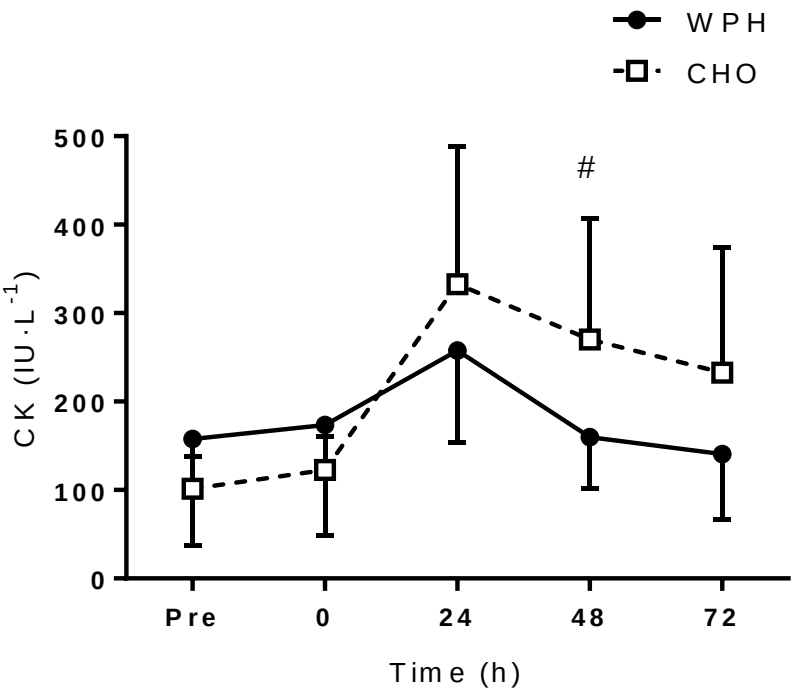

Figure 22. Total creatine kinase (CK) post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (*n* = 10) and carbohydrate (CHO) (*n* = 10) groups. Values presented as mean ± SD. #denotes significantly greater reductions at 48 h in WPH group. Significance at the p < 0.05.

Table 16. Values for dependent variables in response to muscle-damaging exercise, mean ± SD.

Variable	Group	Time post muscle-damaging exercise (h)				
		Pre	**0**	**24**	**48**	**72**
DOMS, mm	WPH	0.0 ± 0.0	16.8 ± 19.9	47.6 ± 26.7	56.7 ± 17.8	19.4 ± 13.2
	CHO	1.0 ± 2.5	13.0 ± 20.1	65.0 ± 49.0	71.2 ± 45.0	37.1 ± 27.4
RF PPT, % (N)	WPH	100 ± 0 (61.1 ± 18.2)	102.5 ± 13.0 (63.8 ± 25.0)	89.9 ± 16.6 (56.1 ± 23.7)	98.9 ± 14.7 (62.0 ± 24.8)	120.5 ± 23.2 (75.3 ± 30.8)
	CHO	100 ± 0 (52.6 ± 14.7)	102.3 ± 11.2 (53.8 ± 15.3)	97.4 ± 30.6 (51.7 ± 23.8)	104.1 ± 29.2 (55.4 ± 23.0)	123.4 ± 36.1 (65.6 ± 26.7)
VL PPT, % (N)	WPH	100 ± 0 (61.0 ± 17.5)	101.5 ± 12.0 (61.9 ± 20.2)	87.4 ± 15.7 (53.9 ± 20.4)	95.5 ± 20.7 (59.1 ± 23.9)	119.5 ± 18.2 (73.7 ± 26.8)
	CHO	100 ± 0 (50.9 ± 15.6)	99.5 ± 12.5 (50.3 ± 15.2)	98.2 ± 25.9 (48.7 ± 16.6)	100.7 ± 33.0 (50.9 ± 21.6)	120.8 ± 37.2 (60.8 ± 24.8)
GM PPT, % (N)	WPH	100 ± 0 (60.6 ± 20.4)	101.1 ± 15.6 (61.1 ± 23.5)	94.1 ± 16.6 (57.2 ± 22.2)	106.9 ± 15.1 (64.1 ± 21.7)	125.9 ± 22.5 (74.2 ± 23.0)
	CHO	100 ± 0 (48.6 ± 17.8)	97.3 ± 15.5 (47.2 ± 17.5)	94.6 ± 26.3 (45.7 ± 21.0)	101.9 ± 28.8 (48.3 ± 17.8)	116.0 ± 28.5 (56.0 ± 24.4)
Thigh girth, % (cm)	WPH	100 ± 0 (51.9 ± 4.4)	100.3 ± 0.8 (52.1 ± 4.4)	100.1 ± 0.6 (52.0 ± 4.3)	99.8 ± 1.2 (51.8 ± 4.0)	99.7 ± 1.0 (51.7 ± 4.2)
	CHO	100 ± 0 (48.9 ± 3.5)	99.9 ± 0.7 (48.8 ± 3.3)	100.2 ± 0.8 (48.8 ± 3.2)	100.2 ± 0.8 (48.8 ± 3.2)	100.6 ± 0.5 (48.8 ± 3.3)
Calf girth, % (cm)	WPH	100 ± 0 (36.9 ± 1.8)	99.9 ± 0.4 (36.8 ± 1.7)	99.6 ± 0.5 (36.7 ± 1.7)	99.8 ± 0.6 (36.8 ± 1.7)	99.9 ± 0.8 (36.8 ± 1.7)
	CHO	100 ± 0 (35.0 ± 2.8)	99.6 ± 0.5 (34.9 ± 2.7)	99.7 ± 1.1 (34.9 ± 2.7)	99.7 ± 0.6 (34.9 ± 2.8)	100.0 ± 0.9 (35.0 ± 2.9)

Table 16. Continued

Variable	Group	Time post muscle-damaging exercise (h)				
		Pre	**0**	**24**	**48**	**72**
Flexibility, % (cm)	WPH	100 ± 0 (28.1 ± 5.8)	100.2 ± 4.0 (28.2 ± 6.2)	92.0 ± 13.7 (26.1 ± 7.3)	83.2 ± 11.9 (23.4 ± 5.7)	106.4 ± 5.7 (29.8 ± 6.2)
	CHO	100 ± 0 (24.8 ± 7.6)	89.8 ± 15.6 (23.0 ± 8.8)	84.4 ± 16.3 (21.4 ± 8.4)	78.2 ± 26.7 (20.6 ± 10.1)	85.6 ± 22.6 (22.1 ± 9.9)
CMJ, % (cm)	WPH	100 ± 0 (26.8 ± 4.4)	86.7 ± 8.4 (23.2 ± 4.5)	94.2 ± 8.3 (25.3 ± 5.5)	92.2 ± 4.2 (24.6 ± 4.1)	95.2 ± 7.1 (25.6 ± 5.3)
	CHO	100 ± 0 (24.3 ± 2.8)	88.1 ± 6.9 (21.3 ± 2.0)	87.4 ± 10.0 (21.1 ± 2.6)	89.7 ± 9.3 (21.7 ± 2.8)	94.5 ± 11.1 (22.9 ± 3.2)
RSI, % ($cm \cdot s^{-1}$)	WPH	100 ± 0 (114.9 ± 29.9)	88.5 ± 12.4 (102.2 ± 31.0)	89.0 ± 12.6 (101.5 ± 26.4)	89.2 ± 10.4 (102.7 ± 29.2)	94.0 ± 11.3 (108.9 ± 32.8)
	CHO	100 ± 0 (94.9 ± 5.5)	81.3 ± 9.2 (77.0 ± 8.4)	80.4 ± 11.5 (76.1 ± 10.0)	81.6 ± 7.2 (77.3 ± 7.2)	85.3 ± 10.0 (80.9 ± 10.2)
MVC, % (N)	WPH	100 ± 0 (445.0 ± 69.9)	91.6 ± 8.2 (409.4 ± 80.3)	89.4 ± 10.3 (398.2 ± 75.1)	89.5 ± 8.5 (399.8 ± 79.2)	95.0 ± 9.9 (423.7 ± 84.0)
	CHO	100 ± 0 (400.4 ± 66.6)	84.6 ± 7.0 (399.2 ± 68.8)	87.5 ± 9.2 (349.5 ± 61.2)	88.1 ± 8.3 (353.2 ± 70.5)	89.6 ± 11.5 (356.7 ± 62.9)
30 m sprint time, % (s)	WPH	100 ± 0 (5.31 ± 0.34)	102.7 ± 4.5 (5.45 ± 0.38)	101.8 ± 3.5 (5.40 ± 0.37)	101.2 ± 2.8 (5.37 ± 0.38)	99.7 ± 3.4 (5.29 ± 0.36)
	CHO	100 ± 0 (5.36 ± 0.26)	102.7 ± 4.7 (5.50 ± 0.34)	102.7 ± 4.4 (5.50 ± 0.30)	100.6 ± 7.3 (5.38 ± 0.38)	100.7 ± 5.5 (5.39 ± 0.30)
CK, $IU \cdot L^{-1}$	WPH	157.9 ± 120.5	173.7 ± 125.5	257.6 ± 103.6	159.8 ± 58.4	140.7 ± 73.8
	CHO	101.6 ± 36.5	122.8 ± 38.3	332.6 ± 155.9	270.0 ± 137.0	232.5 ± 141.5

WPH, whey protein hydrolysate group (n = 10); CHO, carbohydrate group (n = 10); %, % change from pre-exercise (Pre); DOMS, delayed onset muscle soreness; RF, rectus femoris; VL, vastus lateralis; GM, medial head of the gastrocnemius; PPT, pain pressure threshold; CMJ, countermovement jump; RSI, reactive strength index; MVC, maximal voluntary isometric contraction; CK, creatine kinase.

6.4 Discussion

This investigation examined the effect of whey protein hydrolysate (WPH) supplementation on exercise recovery following EIMD in females. This study demonstrated for the first time that WPH reduces circulating CK, attenuates the decline in RSI, and accelerates recovery of hamstring flexibility compared to isocaloric CHO supplementation following repeated-sprint exercise in female dancers.

While not all measures improved, this study is in agreement with a number of investigations reporting accelerated recovery of muscle functionality following EIMD with ingestion of WPH (Buckley et al., 2010; Cooke et al., 2010; Hansen et al., 2015); although some have demonstrated no effect (Farup et al., 2014; Rahbek et al., 2015), or in fact a detrimental effect (Lollo et al., 2014). Indeed, one study observed that isometric muscle force recovered beyond baseline values by 6 h post EIMD after a single 25 g dose of WPH, while it remained suppressed with isoproteic whey protein isolate and non-caloric placebo supplementation (Buckley et al., 2010). The predominant mechanism thought to be responsible for the role of WPH in accelerating recovery is through the provision and increased availability of amino acids; vital for regeneration and/or *de novo* synthesis of protein and the repair of damaged contractile elements of the muscle fibres (Biolo, Tipton, Klein, & Wolfe, 1997). Indeed, WPH supplementation may be superior compared to other forms of protein in this regard, as plasma concentrations of amino acids and dipeptides (and therefore their bioavailability) are greater following ingestion of protein hydrolysates compared to non-hydrolysed proteins (Koopman et al., 2009; Morifuji et al., 2010; Power et al., 2009; Tang et al., 2009). Importantly, while global MPS is increased with dietary protein intake, this includes an increase in myofibrillar protein synthesis observed at rest (Brodsky et al., 2004), and following resistance (Moore et al., 2009), endurance (Breen et al., 2011), concurrent (Camera et al., 2015), and repeated-sprint cycling exercise (Coffey et al., 2011). Myofibrillar proteins may be damaged during eccentric contraction, as sarcomeres are ‘overstretched’ beyond filament overlap. This has been associated with a subsequent increase of sarcomeres in series, and therefore a shift of the length-tension

relationship to the right; towards longer muscle lengths with an increase in optimal angle for force generation (Morgan & Allen, 1999; Philippou, Bogdanis, Nevill, & Maridaki, 2004; Proske & Morgan, 2001). An increase in myofibrillar protein synthesis with WPH ingestion may contribute to repair and remodeling of damaged myofibrils and accelerate the addition of sarcomeres in series following EIMD; allowing for the muscle fibres to work at longer lengths. Perhaps this may explain the observed improvement in hamstring flexibility with WPH. The reduction in CK at 48 h post EIMD with WPH supplementation reported in the present study also lends support to a potential acceleration of myofibrillar repair.

In addition, more compliant muscles are thought to be capable of storing more elastic energy (Brughelli & Cronin, 2007), therefore performance during activities utilising the stretch shortening cycle (such as drop jumps for measurement of RSI) may be improved. However, reductions in CK and improvements in flexibility were only evident at 48 h and 72 h post exercise respectively, while reductions in RSI were attenuated throughout recovery. Notwithstanding, no other measures of muscle function were effected by WPH supplementation. Therefore, the role of sarcomereogenesis in attenuating increases in CK and reductions in RSI, and accelerating recovery of flexibility with WPH supplementation remains speculative and warrants further investigation.

To date, two studies that investigated the supplementation of WPH post EIMD have examined the potential cellular mechanisms responsible for promoting regenerative processes and influencing the rate of recovery (Farup et al., 2014; Rahbek et al., 2015). Rahbek et al. (2015) examined the signaling associated with muscle protein turnover post EIMD with supplementation of 84 $g \cdot day^{-1}$ WPH with 84 $g \cdot day^{-1}$ carbohydrate (WPH-CHO) for three days (divided into three equal portions) compared to isocaloric CHO in recreationally active males. The authors reported an increase in phosphorylation of mechanistic target of rapamycin (mTOR), ribosomal protein S6 kinase beta-1 (p70S6K) and ribosomal protein S6 (rpS6), and a decrease in phosphorylation of forkhead box O1 (FOXO1) and forkhead box O3 (FOXO3) in an eccentrically induced muscle damaged leg, with no group differences between supplements. However, interaction effects demonstrated that phosphorylation of Akt kinase was lower in the exercised leg, and phosphorylation of FOXO1 was higher in

the control leg following WPH-CHO compared to isocaloric CHO. Interestingly, these changes in signaling pathways were not correlated with rate of muscle force recovery and there was in fact an increase in muscle soreness with WPH-CHO supplementation. However, a similar study from the same laboratory demonstrated that WPH-CHO supplementation accelerated satellite cell (SC) proliferation (notably in type II fibres) compared to an isocaloric CHO (Farup et al., 2014). Given that SC are essential for regeneration of skeletal muscle (Relaix & Zammit, 2012), this suggests that WPH-CHO supplementation might increase repair and remodeling processes following EIMD. Yet, as in the aforementioned study, the increased SC proliferation did not translate to improved recovery of muscle function (Farup et al., 2014). Despite these studies failing to observe improvements in muscle function, the myocellular effects that were reported are nevertheless thought to contribute to the repair of damaged muscle. It is therefore conceivable that these present potential mechanisms responsible for the reduction in force loss of RSI and an accelerated recovery of hamstring flexibility with WPH supplementation in the current investigation; however, this remains to be explicitly demonstrated.

It is important to note that the majority of previous investigations examining the efficacy of WPH for exercise recovery have included substantial carbohydrate supplementation (Cooke et al., 2010; Farup et al., 2014; Hansen et al., 2015; Rahbek et al., 2015). This makes it difficult to determine the contribution of the WPH alone to their observations. It has been reported that while MPS is unaffected, carbohydrate ingestion attenuates MPB following exercise (Børsheim et al., 2004; Miller, Tipton, Chinkes, Wolf, & Wolfe, 2003). For instance, the addition of carbohydrate to an amino acid mixture failed to result in increased MPS following resistance exercise compared to amino acids alone (Miller et al., 2003). Indeed, co-ingestion of carbohydrate during recovery does not further increase MPS when protein is ingested in adequate quantities (Koopman et al., 2007; Staples et al., 2011). Therefore, given that protein intakes appeared to be sufficient in the aforementioned WPH studies, the beneficial effects were likely due to the amino acid provision as opposed to other ingredients in the supplement. However, given the increased energy associated with additional carbohydrate, consumption of WPH alone might be favoured by populations concerned with total energy intake, such as female dancers in the current study.

A strength of the present investigation was the dietary control employed throughout testing periods. The female dancers either achieved the recommended 1.2-1.7 $g \cdot kg^{-1} \cdot day^{-1}$ of protein (Tipton & Wolfe, 2004) (CHO group; 1.3 ± 0.2 $g \cdot kg^{-1} \cdot day^{-1}$) or a protein-rich diet (WPH group; 1.8 ± 0.2 $g \cdot kg^{-1} \cdot day^{-1}$). Although the debate remains, some argue that as long as recommended levels of protein are achieved, further supplementation might be unnecessary in trained populations (Tipton, 2008). Despite this, a number of well-controlled studies have demonstrated that WPH (Hansen et al., 2015; Lollo et al., 2014) and BCAA (Coombes & McNaughton, 2000; Jackman et al., 2010) supplementation is beneficial in attenuating EIMD, in spite of participants consuming recommended protein intakes. In the present investigation, since both groups were provided with sufficient intakes of macronutrients, and the daily diet and supplements were isocaloric, the attenuated reductions in muscle function and lower CK can be attributed to the additional protein provided by the WPH. Therefore, this study lends support for the use of additional protein beyond recommended levels to reduce muscle damage and accelerate recovery following strenuous exercise.

Total energy intake appears to influence protein synthesis which might be inhibited by energy depletion at the cellular level (Kumar et al., 2009). Recent research demonstrates that MPS is down-regulated when in energy deficiency and as a result, energy deficient individuals should consume high protein diets (1.6-2.4 $g \cdot kg^{-1} \cdot day^{-1}$) to restore MPS and attenuate proteolysis and skeletal muscle loss (Pasiakos et al., 2013; Pasiakos et al., 2015). A number of studies have determined that dance populations are (for the most part) in negative energy balance or have low energy availability (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Doyle-Lucas et al., 2010; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002). This might explain why the female dancers supplemented with WPH beyond recommended levels experienced ameliorated recovery from EIMD. Indeed, some research indicate that protein supplementation might elicit greatest ergogenic effects for individuals in negative protein and/or energy balance (Pasiakos et al., 2014).

There are a number of limitations associated with this study that warrant acknowledgement. Firstly, this study did not measure nitrogen balance, signaling

enzymes associated with protein turnover, nor rates of MPS and MPB. Therefore, it was not possible to identify specific mechanisms, which might have been responsible for the attenuated muscle damage response and accelerated recovery from EIMD with WPH compared with isocaloric CHO. Moreover, besides the provision of amino acids, there may be other mechanisms by which WPH influences recovery from EIMD. For instance, protein hydrolysates have been reported to exhibit antioxidant properties (Peng, Xiong, & Kong, 2009) which might contribute to reducing muscle damage by attenuating the oxidative stress response associated with strenuous exercise. Moreover, WPH dipeptides have also been shown to increase glucose uptake in isolated skeletal muscle (Morifuji, Koga, Kawanaka, & Higuchi, 2009). While not measured in the present investigation, such effects of WPH might certainly have contributed to the present findings. In addition, this study was limited by the absence of further treatment groups; for instance, a non-caloric control, a group matched for carbohydrate content, and an isoproteic group, which consisted of whole or intact protein. This would have allowed further comparisons regarding the efficacy of a divergence of nutritional strategies. Indeed, the CHO group in the present investigation consumed significantly greater carbohydrate compared to WPH group. While both groups consumed recommended post-exercise and daily carbohydrate intakes, and both supplements and daily dietary intakes were isocaloric, higher carbohydrate intakes might have masked differences between groups. Moreover, it can only be speculated that the observed improvements with WPH were as a result of the partially digested form of protein rather than other protein sources. The intervention in the present study also involved ingestion of WPH immediately post EIMD, and throughout the recovery period; therefore, it is difficult to identify whether ingestion close to the exercise bout is important. Interestingly, while RSI was significantly higher with WPH supplementation compared to an isocaloric CHO throughout recovery, the decline in RSI immediately post-exercise and ingestion of the first supplement was not different between groups (11.5 ± 12.4 and 18.8 ± 9.2% in WPH and CHO groups, respectively; independent samples *t* test; $p = 0.155$). In addition, the interaction effects observed in measures of CK and flexibility were evident at 48 h and 72 h post EIMD, respectively. Intuitively, for optimal recovery amino acids should be ingested both immediately and in the days of recovery post-exercise where MPS is thought to persist (Phillips et al., 1997). However, the present study did not investigate the influence of

supplementation timing and more research is warranted to establish optimal supplementation strategies. Finally, it is also important to note that the resultant amino acids and peptides following hydrolysis can be of varying size depending on conditions during manufacturing; namely on the method and duration of hydrolysis. Therefore since the specific properties of protein hydrolysates are mediated by the manufacturing process (Thomson & Buckley, 2011) and as the end product is rarely reported in studies, this limits our understanding of their efficacy.

6.5 Perspectives

This chapter addressed the fourth aim of the thesis: *'to investigate the influence of whey protein hydrolysate supplementation on exercise-induced muscle damage in female dancers.'* The results from this study resulted in the rejection of the null hypothesis, concluding that whey protein hydrolysate supplementation had a significant influence on exercise-induced muscle damage in female dancers. This study sought to elucidate whether the benefits of WPH which have been reported previously can be demonstrated in female dancers following a repeated-sprint exercise bout. The main findings of this study were that four days of WPH supplementation improved recovery of muscle function (evidenced by improved RSI and flexibility) compared to isocaloric CHO supplementation, and that this was likely attributable to a reduction in muscle damage (evidenced by reduced CK). Though not directly measured, it is also likely that an increased delivery of amino acids with WPH supplementation was responsible for accelerating the repair of damaged skeletal muscle and thus its force generating capacity. While the observed improvements are arguably modest, acceleration in recovery of muscle function is of relevance to female dancers who are expected to perform daily, and therefore is an important consequence of WPH supplementation. Indeed, these data support previous research demonstrating that protein intakes beyond recommended levels can ameliorate recovery from EIMD. This research adds to the existing body of knowledge indicating benefits of WPH, whilst providing new information for the novel application in wider populations. Specifically, to female dancers who are at risk of symptoms associated with muscle damage, and would benefit from a practical nutritional intervention to both improve recovery on subsequent days, and

contribute to restoring energy balance. Therefore, in regards to practical implications for healthcare professionals working with dancers (please refer to section 7.2 for more details), these findings suggest that WPH can be advised for accelerating recovery from EIMD in female dancers; specifically following strenuous exercise. It is also likely to be a beneficial intervention during intensified training periods, where recovery times may be limited.

7 General discussion

The aims of this thesis were to increase knowledge and understanding of the nutritional status and the exercise recovery response of female dancers. More specifically, this thesis sought to determine 1) the exercise and eating behaviours of female dancers during full-time dance training; 2) the physiological and functional response of female dancers to strenuous exercise; and 3) the efficacy of two nutritional interventions in reducing the symptoms of EIMD and accelerating recovery in a female dance population. In order to address these research questions, associated statistical null hypotheses were formulated. The results of this course of investigation resulted in these null hypotheses being rejected, concluding that 1) there is a difference between energy intake and energy expenditure of pre-professional female dancers; 2) both dance-specific and repeated-sprint exercise results in EIMD in female dancers; 3) Montmorency tart cherry juice (though a small effect) and 4) whey protein hydrolysate supplementation accelerate recovery in female dancers. This chapter will synthesise the main findings of the thesis in the context of existing literature, highlight the limitations of the work, provide recommendations for the female dance population, and identify potential future research directions.

7.1 Synopsis of experimental chapters

The first experimental study (chapter 3) sought to determine the typical activity and eating behaviours of pre-professional female dancers. Given the importance of maintaining a lean and aesthetic physique in dance and the previously reported training schedule of dance populations, it was hypothesised that the dancers would be in energy deficit. As anticipated, the data demonstrated that during a 7-day period this population were in a negative energy balance (-356 ± 668 kcal·day^{-1}) and had a low energy availability (26 ± 13 kcal·kg FFM^{-1}·day^{-1}). Though not directly measured, the dancers could be at risk of disorders associated with energy imbalance. Interestingly, while exercise activity remained unchanged, eating behaviours varied between working week days (where participants had scheduled dance training) and the weekend. The percentage of total energy intake (%TEI) derived from fat and alcohol was higher, and %TEI from carbohydrate was lower at the weekend. As a result, total energy intake (TEI) was higher and energy balance

was in fact slightly positive during this period (123 ± 1007 kcal·day^{-1}). Perhaps the dancers perceived that while they were not in training they could indulge in arguably less desirable nutritional behaviours, or alternatively compensate for the low energy intakes during the week.

Whilst the observation technique is considered the gold-standard method for determination of energy intake, this method is neither realistic nor appropriate for use in free-living conditions. Therefore, study 1 in this thesis used a new approach to examine free-living TEI in this population by combining self-reported weighed food diaries with 24 h recall interviews. This method has demonstrated good agreement with the gold standard observed food intake technique in adolescent athletes (Briggs, Rumbold, et al., 2015; Rumbold et al., 2011). It is important to note that the accuracy of this technique has not been established in this specific population. Moreover, the use of biochemical measures of nutritional status (such as serum/plasma concentrations of water and fat soluble vitamins and trace elements) to substantiate these data would have strengthened the study findings. Having said this, while biomarkers offer a more objective assessment of nutritional status, these methods are costly and are subject to large individual variability, and the rapid turnover of micronutrient concentrations limits their sensitivity (Elmadfa & Meyer, 2014). Therefore, development of the current analysis procedures is warranted to advance the accuracy and precision of nutritional biomarkers for use in future research.

As with energy intake, it is also imperative that exercise energy expenditure is measured in a free-living environment to characterise typical behaviours. While the direct/indirect calorimetry techniques are considered to be precise and accurate in assessment of all components of total energy expenditure, these were not employed given their restrictive nature. This study utilised tri-axial accelerometry to assess exercise energy expenditure, and combined this with estimated basal metabolic rate and the thermic effect of food to estimate total energy expenditure (TEE). While there are limitations associated with these assessment techniques (previously discussed in section 2.1.4), this is the first study to consider all aspects of TEE in female contemporary dancers. The findings are in line with a number of investigations reporting that dancers are typically in negative energy balance or low energy availability (Beck, Mitchell, et al., 2015; Dahlstrom et al., 1990; Doyle-

Lucas et al., 2010; Hassapidou & Manstrantoni, 2001; Hirsch et al., 2003; Hoch et al., 2011; Kostrzewa-Tarnowska & Jeszka, 2003; Robbeson et al., 2015; Warren, Brooks-Gunn, et al., 2002). There is certainly a growing body of evidence demonstrating an unsettling prevalence of poor nutrition in dancers. This could have substantial implications on many aspects of dance performance, health and well-being, and as a result there is considerable need to address energy imbalance in these populations.

Owing to the demands of dance training (discussed in section 2.2.4), female dancers may be at risk of experiencing symptoms associated with EIMD. However, prior to this course of investigation, only one study (Rodrigues-Krause et al., 2014) has sought to investigate muscle damage following dance activity and there were a number of limitations; namely in the study design (discussed in section 2.2.4). The second experimental study (chapter 4) directly examined the EIMD response and subsequent recovery following dance-specific exercise (DP protocol) in female dancers, and compared the profile of damage to a more traditional sport-specific exercise model (SP protocol). The findings demonstrated that DOMS, limb girth and plasma CK increased and muscle function was reduced following the DP, and that symptoms were evident for several days. In agreement with others (Leeder et al. (2014), Howatson et al. (2010), and Bell et al. (2016), for instance), EIMD was observed in spite of the participants being trained and accustomed to the exercise activity. Additionally, while there was some variation in the EIMD response (interaction effects for thigh girth and CMJ), there were no group differences between the dance and sprint-specific protocols; demonstrating that the physiological profiles following damage and during recovery were similar. These data substantiate previous work indicating that dance activity (ballet class and rehearsal) increases systemic indices of muscle damage (Rodrigues-Krause et al., 2014) and that this occurs with concomitant increases in muscle soreness, and reductions in muscle function. Notwithstanding, for the first time, these findings demonstrate that muscle damage is experienced following dance activity representative of contemporary dance.

Arguably, dance has traditionally been perceived to be a recreational activity as opposed to a sport, and so the implications of such activity have been largely overlooked - as evidenced by the scarcity of research available in this area.

However, while there are distinct differences between sport and dance, for instance in the motivation and outcomes of performance, it has long been argued that there are also many similarities between these activities (Ingram, 1978). Parallels are perhaps most evident with aesthetic sports such as figure skating, rhythmic gymnastics, and synchronized swimming which are judged to some extent on movement quality, creativity, or style (Kleinman, 1992). Dancers and other athletes each use the body as an 'instrument', each are entertaining to watch and attract spectators, and the processes and skills required to perform (including coordination, endurance, balance, and cardiovascular efficiency) are similar (Ingram, 1978). Indeed, early work reported that the physical demands placed on individuals during ballet were equal, or in fact more demanding compared to a variety of strenuous activities, including basketball, hockey, rugby and soccer (Nicholas, 1975). Certainly, since dancers and other athletes are subject to similar physiological stressors, these populations share many medical concerns (Solomon, Clarkson, Micheli, & Trepman, 2001). In support of these previous works, the evidence presented here suggests that the challenges faced by dancers during recovery are akin to those experienced by other sport-specific activities that precipitate damage. Consequently, this thesis supports the proposal that dancers should be recognised as performing/aesthetic/artistic athletes (Angioi, Metsios, Koutedakis, & Wyon, 2009; Koutedakis & Jamurtas, 2004).

Chapters 3 and 4 provided an important foundation for subsequent studies in the present course of investigations. The latter provided objective evidence that interventions designed to attenuate EIMD are warranted in female dancers. The preceding evidence of negative energy balance in this population indicated that investigation of the efficacy of nutritional interventions would be meaningful. Certainly, nutritional strategies might prove to be beneficial both in accelerating exercise recovery and in contributing to the restoration of energy balance in this population. Nutritional interventions remain among the most commonly used strategies to enhance recovery in sport and exercise (Howatson & van Someren, 2008). Whilst adequate intakes of macronutrients, electrolytes and fluids are vital, there may be a role to play for additional nutritional supplementation to further advance the recovery process. In the last decade, the efficacy of functional foods (such as those investigated in the final two studies of this thesis) has been of

particular contemporary interest. These may offer natural alternatives to pharmacological interventions and analgesics, which carry additional risks and potentially harmful side effects (Ziltener et al., 2010). Moreover, the emerging interest in functional foods is perhaps due in part to the suggestion that 10-15% of supplements contain prohibited substances (Outram & Stewart, 2015), and therefore carry an additional risk of contravening doping regulations with their consumption. While the use of such substances in dance populations is not currently controlled under the World Anti-Doping Agency, dancers have been reported to use performance-enhancing substances and are still at risk of suffering adverse effects (Boardley, Allen, Simmons, & Laws, 2016). Consequently, more natural alternatives, which can also offer performance and/or recovery benefits, are preferred and highly sought after. Consequently, the final experimental studies of the thesis (chapters 5 and 6) aimed to explore the efficacy of nutritional interventions on EIMD in female dancers.

Tart Montmorency cherries (MC) have been reported to be an effective recovery aid due to the high anti-inflammatory properties and antioxidant content present within them (Bell, Walshe, et al., 2014; Bell et al., 2015; Keane, Bell, et al., 2015; Kirakosyan et al., 2015; Seeram et al., 2001; Wang, Nair, Strasburg, Chang, et al., 1999). A number of studies have now demonstrated positive effects of MC supplementation on recovery following damaging exercise (Bell et al., 2016; Bell, Walshe, et al., 2014; Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010; Kuehl et al., 2010; Levers et al., 2015). However, whether the benefits previously reported in male and mixed-sex populations are also observed in females has not yet been determined. Consequently, the third experimental study aimed to build upon the current evidence by examining the efficacy of MC supplementation following EIMD in female dancers. Given that chapter 4 determined that no group differences in EIMD and recovery between the DP and SP were evident, this study (and the final study) employed the SP protocol as an appropriate model to induce muscle damage in female dancers. Indeed, a recent study has reported reductions in DOMS, inflammation, and accelerated recovery of muscle function with MC following an adapted LIST protocol (repeated, intermittent-sprint exercise protocol) in semi-professional male soccer players (Bell et al., 2016).

The main finding of chapter 5 was that 8-day MC supplementation improved recovery of muscle function (CMJ) compared to PL, in agreement with the literature to date (Bell et al., 2016; Bell et al., 2015; Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010). Moreover, muscle soreness tended to be lower compared to PL; also observed previously by others (Bell et al., 2016; Connolly et al., 2006; Kuehl et al., 2010; Levers et al., 2015). Given that circulating concentrations of CK and hsCRP were not different between treatment groups, it is likely that the observed effects could be attributable to reductions in oxidative stress (or perhaps alternative unmeasured mechanisms) rather than a loss of sarcolemmal integrity and inflammation. This idea is consistent with Bowtell et al. (2011), who reported attenuated concentrations of protein carbonyls with MC compared to PL supplementation in the absence of differences in CK and hsCRP. However, given that antioxidant capacity and oxidative stress were not directly measured in the present thesis, this remains speculative. Importantly, while not all measures were favourably affected, the additional energy provided by MC supplementation (204 kcal·day^{-1}) would also improve the energy imbalance of female dancers, shown to be potentially detrimental to health and well-being. Certainly, cherries are considered to be a nutrient dense food, with significant amounts of bioactive food components, with a relatively low caloric content (McCune et al., 2011). Therefore, though potential effects for exercise recovery may be small, this may represent an attractive nutritional strategy to ameliorate symptoms of EIMD in this population who are characteristically concerned with energy intake.

The final experimental study (chapter 6) sought to investigate supplementation with whey protein hydrolysate (WPH); another nutritional intervention previously demonstrated to improve recovery from EIMD (Buckley et al., 2010; Cooke et al., 2010; Farup et al., 2014; Hansen et al., 2015; Lollo et al., 2014; Rahbek et al., 2015). Certainly, the repair of skeletal muscle which is vital for maximising recovery requires a positive net protein balance (Hawley et al., 2006; Saunders, 2007; Tipton, 2008; Tipton & Wolfe, 2001) and this may only be achieved post exercise with sufficient protein ingestion (Kumar et al., 2009; Phillips et al., 1997; Pitkanen et al., 2003). However, no study has yet investigated the efficacy of WPH in reducing EIMD beyond a model of isolated eccentric contractions, or a long-term training programme. Additionally, as with the MC literature, none have recruited a

female only population. Therefore, in light of these limitations, chapter 6 sought to examine the influence of WPH supplementation on EIMD and recovery in female dancers.

This study demonstrated that 4-day WPH supplementation reduced circulating CK, attenuated the decline in RSI, and accelerated the recovery of hamstring flexibility compared to isocaloric CHO supplementation following repeated-sprint exercise in female dancers. Notably, this was observed in spite of robust dietary control enforced throughout trial periods, whereby all participants received carbohydrate (5-7 $g \cdot kg^{-1} \cdot day^{-1}$) (Burke et al., 2006) and protein (1.2-1.7 $g \cdot kg^{-1} \cdot day^{-1}$) (Tipton & Wolfe, 2004) intakes that are recommended for athletic populations. Interestingly, while CK was reduced, hsCRP was not influenced by WPH; suggesting that diminished inflammation was not responsible for the observed improvements. This is in line with studies conducted by Hansen et al. (2015) and Buckley et al. (2010) who reported accelerated recovery of muscle function in the absence of changes in a variety of systemic inflammatory indices. Though not directly measured, it is likely that exogenous delivery of amino acids improved protein balance and increased MPS; accelerating the repair of damaged tissue. Indeed, Rahbek et al. (2015) reported alterations in Akt-mTOR and FOXO signalling proteins, and Farup et al. (2014) reported increased SC proliferation with 3-day supplementation of WPH with CHO compared to isocaloric CHO. As with MC, the ingestion of WPH in the present thesis (176 $kcal \cdot day^{-1}$) is also expected to improve energy balance in female dancers. Certainly, recent evidence suggests that protein requirements are increased when in energy deficiency (Pasiakos et al., 2013; Pasiakos et al., 2015).

Interestingly, recent studies have reported that dietary supplement use is prevalent amongst dancers, particularly the use of multivitamins, caffeine, creatine and whey protein (Boardley et al., 2016). These data demonstrate that, as in other athletic populations, dancers seek to support and facilitate their training and performance through supplementation. Despite this, the evidence surrounding the efficacy of these strategies are not well researched in dance populations. Indeed, the aforementioned study identified that dancers' knowledge in the use of nutritional supplementation relies largely on peer recommendation rather than evidence-based advice from health-care professionals and without scientific support. Certainly, studies regarding nutritional intake, energy expenditure, and dietary

recommendations for dancers are scarce (Sousa et al., 2013). Consequently, the results of this thesis have wide-reaching applications to dancers (and indeed other athletic populations) where data is currently very limited and there is a need for population specific research. This research suggests that MC and WPH can be recommended as practical and convenient nutritional supplements for female dancers to consume, offering additional calories as well as benefits relating to exercise recovery.

7.2 Implications and practical recommendations for healthcare professionals

According to the Sport and Recreation Alliance (2014) there are 5.5 million adults recreationally participating in dance-type activity in the United Kingdom alone. These activities include (but are not limited to) fitness and group exercise such as dance aerobics and Zumba®, and more traditional forms of dance such as ballet, breakdance, ballroom, and contemporary dance. As well as recreational dance activity, the 1909 Syllabus of Physical Training (Ministry of Education, 1911) established dance in the school curriculum, and its importance in education has persisted. As a constituent of physical education taught in the national curriculum at key stage 1 to 4, dance performance is offered by 96% of schools (Quick, Simon, & Thornton, 2010) and can also be studied at all subsequent levels of education. With 25 professional schools and colleges offering dance training courses accredited by the Council for Dance Education and Training, and universities offering a great number of dance related programmes, there are an estimated 1,000 students graduating from dance courses each year (Dance UK, 2015). The scope of the professional dance industry in the United Kingdom is also vast; with dancers performing in theatres such as in West End musicals, on cruise ships, as dance educators, and as cast members in an estimated 200 dance companies (Dance UK, 2015). These data demonstrate the popularity and scale of dance participation and the potential expansive impact that dance-related research can achieve both in the national and international dance communities.

Certainly, this particular research suggests (within the limitations outlined in each experimental chapter and in the following section of this chapter) that there are many dancers who could be at risk of a number of health and performance related

impairments, and as a result may seek professional guidance. Consequently, some practical recommendations can be offered to healthcare professionals, based on the current thesis findings. Firstly, given the prevalence of energy deficiency reported in study 1, pre-professional female dancers should be advised about the energy demands of training and performance, the importance of energy balance, and the consequences of poor nutrition (please refer to section 7.4 for discussion of related future directions). Secondly, dancers should be made aware of the risk of EIMD and its associated symptoms when taking part in dance or repeated-sprint exercise. Specifically, this research demonstrated that symptoms were evident following novel and unaccustomed exercise representative of contemporary dance in female recreational dancers. However, in light of previous evidence of muscle damage following ballet (Rodrigues-Krause et al., 2014), it makes the expectation tenable that other styles of dance might also elicit a muscle damage response (though this remains to be determined). Finally, healthcare professionals and other professionals working with dancers should advise that nutrition can aid in the recovery from muscle damage. More specifically, evidence from this course of investigation suggests that both MC and WPH are effective to some extent at improving symptoms of damage in female recreational dancers. Though not directly compared, WPH supplementation appeared to attenuate the damage response to a greater extent compared to MC supplementation. These nutritional strategies might be of most benefit in periods of high training load and short recovery times, and could play a role in improving adherence to training and performance capacity in the ensuing days. Indeed, optimal recovery and maintaining an ability to perform on a daily basis is often the primary goal for dancers.

7.3 Limitations

The limitations associated with each study are discussed in each respective experimental chapter. However, there are overarching limitations related to this course of investigation that warrant acknowledgement. Specifically, these relate to the ecological validity of the findings. The research design and experimental controls employed throughout the studies allowed for robust analysis of the research questions, however in some instances, this was at the expense of a degree of

ecological validity. These are explored here in the context of the delimitations of the current work.

Firstly, the inherent limitations of the study designs employed in this course of investigation should be acknowledged. Study 1 was a cross-sectional study, investigating eating and exercise behaviours of pre-professional dancers during a 7-day period towards the end of their academic year. By their very nature, trends in chronic TEI and TEE cannot be described from cross-sectional studies. Indeed, the observed behaviours are likely to differ if measured at different times of year; for example, at the beginning of the academic year when pre-professional dancers are likely less fatigued. Consequently, future research should investigate trends in the eating and exercise behaviours of dancers over time in longitudinal studies. However, if repeated at different periods, studies should look to include the same participants across these time points, in order to assess changes that are a true reflection of trends rather than of different samples. Given the restricted time-frame of data collection, cross-sectional studies are also prone to non-response bias, resulting in a sample that may not be representative of the population (Sedgwick, 2014). For that reason, and as previously recommended (Mann, 2003), efforts were made to maximise the number of responders in study 1 within time constraints. Moreover, important advantages of this cross-sectional study were that it was a relatively inexpensive and time-effective means of collecting data, with a reliance on few resources, and low participant burden (with no follow up). Indeed, despite the aforementioned limitations, cross-sectional study designs are appropriate for estimating prevalence of behaviours in a population, which can then be more rigorously studied in randomised controlled trials (RCTs).

While recruited participants in studies 2-4 were randomly assigned to one of two treatments (and stratification ensured that these were matched and counterbalanced for muscle function), these studies were not registered as RCTs. This is because convenience samples were recruited, and as a result, application of the findings to the wider target population of female recreational dancers should be done with caution (Kendall, 2003). Indeed, a widespread criticism of RCTs is that though these are associated with robust internal validity, these are still susceptible to limited external validity. For instance, the difference between the trial protocol and real-world practice will certainly undermine external validity (Rothwell, 2006).

Moreover, in studies 2 and 4, true controls (a 'negative' control, or indeed no intervention) were not used; rather appropriate comparative interventions were used (repeated-sprint protocol and isocaloric carbohydrate, respectively). The use of such 'active' or 'positive' controls has been criticised, with reports that the lack of a 'zero point' reference mark means that differences between treatments cannot be effectively detected (Temple & Ellenberg, 2000). However, it is also argued that this is a credible approach to study design, and it is more useful (and indeed more ethical) to compare an intervention to another established intervention, to determine how these differ or indeed elucidate which of these is superior (Nardini, 2014). Nevertheless, in the hierarchy of evidence, RCTs remain the gold standard when evaluating the effectiveness of an intervention (Barton, 2000). Consequently, future research should employ this study design to provide the strongest evidence for the efficacy of nutritional strategies for reducing EIMD in female dancers.

The lack of literature examining the muscle damage response and the efficacy of nutritional interventions in reducing muscle damage in female participants is surprising, and therefore the results represent a valuable and important contribution to the literature. As discussed previously (section 2.2.3.3), whilst the evidence regarding sex differences in EIMD remains controversial, oestrogen has been implicated to some extent in an attenuated muscle damage response reported in females (Kendall & Eston, 2002; Tiidus et al., 2005). As a result, there is a need to examine oestrogen's effects in much greater detail *in vivo*, as well as investigation of the EIMD, recovery and adaptive responses in differing phases of the menstrual cycle; an area where there is a great deal of contention in the literature. Though the mechanisms by which oestrogen might mitigate muscle damage and/or augment recovery are not clear, it is apparent that potential confounding variables, including menstrual cycle phase and oral contraceptive use, should be accurately reported to assist careful interpretation of results.

All studies relating to the present work required participants to complete a self-reported menstrual cycle questionnaire; in an attempt to report, and where possible control for these variables. As well as the identification of menstrual cycle phase and contraceptive use, this was also used to assign testing days in studies 2, 3 and 4. Evidently, there is potential for participants to misreport this information. Moreover, participants were not excluded based upon contraceptive use (or indeed type of

contraceptive) with the intention of recruiting a heterogeneous (albeit relatively small) sample that was representative of the female population. Certainly, many women take hormonal contraceptives (Stachenfeld & Taylor, 2014). This indicates the high applicability of these findings to the wider female population; where women may be normally menstruating or use any one of a variety of contraceptives. However, it is recognised that not restricting participant eligibility based upon contraceptive use may have influenced the study findings, and indeed a more homogenous sample might have decreased variability in the outcome variables. Urine ovulation prediction tests and systemic indicators (for instance concentrations of oestradiol) can accurately determine menstrual phase. Whilst these methods would have been beneficial to use to establish the most appropriate period for data collection for each individual, these carry additional logistical constraints. Alternatively, it has been suggested that the most effective technique to truly control and isolate the effect of sex hormones in physiological research is the use of a gonadotropin-releasing hormone agonist (Stachenfeld & Taylor, 2014). This temporarily and reversibly suppresses the menstrual cycle, and oestrogens (and/or progestogens) can then be administered in a controlled fashion. However, though this method is safe, it is costly, invasive (and therefore carries additional ethical considerations), and the application of findings to the heterogeneous female population is questionable.

A further limitation relates to the exercise protocols employed in the current work. Following results from the second experimental study (chapter 4), it was determined that both the DP and SP protocols elicited similar levels of muscle damage in female dancers. As a result, it was concluded that either protocol could be used as a model to induce muscle damage in this population. Given the resources required and logistical limitations associated with the DP (not least in the requirement of participants to have learnt the dance sequence and the necessity for adequate and appropriate space), it was decided that the SP would be used for subsequent studies. In comparison, this protocol required no prior commitment from the participants, and was a more feasible protocol when considering time constraints and logistics associated with this work. Moreover, previous work has shown that dance can include elements of sprint activity, and indeed centre floor exercise and stage performance includes sprint-like and power related tasks such as jumps and

travelling steps (Cohen et al., 1982). Multiple repetitions of all out sprinting is thought to challenge energy systems in a manner typical in a variety of sports (Fitzsimons et al., 1993), and the intermittent nature of the SP is also analogous with contemporary dance performance (Wyon, 2005; Wyon et al., 2002). Finally, supplementary training is receiving attention in the dance science literature to improve dancers' physical fitness. This is largely because it is recognised that traditional dance classes and rehearsals are conducted at relatively low intensity and as such, not only fail to prepare the dancer for performance, but do not adequately stress the physiological systems in a way that induces adaptation (Angioi, Metsios, Koutedakis, & Wyon, 2009). Consequently, less traditional methods of training, such as Pilates, yoga, strength training and running (Kozai, 2012) might play a role in promoting adaptions alongside dance specific training. Therefore, information regarding the EIMD response to repeated-sprint exercise certainly has relevance to dancers participating in such activity as part of a supplementary training program. However, it is acknowledged that the SP is constrained to unidirectional sprinting, and does not truly represent the multi-directional change, skill and cognitive elements associated with dance that are likely to affect various aspects of recovery. Moreover, given that the participants are arguably more unaccustomed to this activity, the muscle damage response might be different to that experienced following dance exercise. This is because participants will be more accustomed to the dance-type activity, and may therefore be safeguarded against symptoms of muscle damage due to the RBE or acute adaptive response. Consequently, the application of the present findings in studies 3 and 4 to dance-specific activity should be made with consideration of this limitation.

As described previously (section 2.2.4), dancers engage in many hours of daily training as well as additional fitness training, rehearsals and/or performances (Bronner et al., 2016; Grove et al., 2013; Twitchett et al., 2010; Weiss et al., 2008; Wyon, 2010). These daily demands may be expected for many consecutive weeks during a performance period (Grove et al., 2013). In the current work (chapters 4-6), a single bout of exercise was used to induce damage, and recovery was monitored for several days where participants were required to refrain from strenuous exercise. This provided the experimental control required for robust conclusions regarding the damage response and the efficacy of nutritional interventions to be drawn. However,

it is possible that these findings would be different during true dance training given that it is composed of cumulative bouts of dance activity and short recovery periods. Having said this, it has been shown that eccentric exercise performed with damaged muscles from a previous bout does not exacerbate damage or affect the repair process (Chen, 2003; Chen & Nosaka, 2006; Ebbeling & Clarkson, 1990; Nosaka & Clarkson, 1995).

A final limitation with regards to the ecological validity of the findings from this thesis relates to the dietary restrictions. For 48 h (in study 2) or 24 h (in study 3 and 4) prior to, and for each of the testing days, participants were asked to avoid alcohol, caffeine, nutritional supplements, and any anti-inflammatory drugs or alternative treatments. Moreover, the second experimental study required participants to be fasted for 2 h prior to EIMD, and studies 3 and 4 stipulated an overnight fast of ≥10 h. In true sport and exercise scenarios, these restrictions would not be imposed and indeed athletic populations might be encouraged to use many nutritional interventions in combination with the intention of optimising multiple aspects of performance, recovery and adaptation. Though beyond the scope of this work, the potential interaction and cumulative effects of supplement and macro- and micronutrient intakes on the measures identified in this work are not well understood and are undoubtedly difficult to investigate. Nonetheless, throughout this course of investigation it was important to control for these potential confounding variables and to examine the effects of exercise and/or supplement use in isolation. This offered greater strength to the current study designs and allowed for a robust analysis of the research in question.

Additionally, the final experimental study controlled all dietary intake; providing participants with recommended intakes of protein and carbohydrate. This was to ensure that any affect observed was not related to insufficient macronutrient intakes, and it was anticipated that (given supplements were isocaloric and there were no differences in total daily energy intake) any group differences would be attributable to the WPH supplement. However, it is important to note that the female dancers recruited may not typically consume these quantities of macronutrients and TEI. Certainly, dietary analysis from self-report weighed food diaries demonstrate that TEI, and quantities of carbohydrate ($g \cdot kg^{-1}$ and %TEI) from studies 2 and 3 were lower than that provided in study 4. Moreover, whilst energy expenditure was not

measured in study 4, the current thesis demonstrated that pre-professional dancers are typically in an energy deficit (chapter 3). Research indicates that protein supplementation might elicit greatest ergogenic effects for individuals in negative protein and/or energy balance (Pasiakos et al., 2014). This suggests that the beneficial effects observed with WPH may in fact be magnified with free-living dietary intakes in this population, although this remains to be elucidated. Nevertheless, whilst dietary restrictions presented here are a positive delimitation of the current work in regards to the internal validity of the findings, the effects of supplementation should be considered in the context of the energy and macronutrient state of the individuals.

7.4 Future directions

This series of investigation has raised a number of questions and potential areas of future research. With regards to the energy intake and energy expenditure data collected in pre-professional female dancers (chapter 3), the current thesis has provided rationale for further work to examine nutritional practices and their implications in this and other dance populations. For instance, as well as impaired physical performance and recovery, energy imbalance and/or low energy availability has been associated with medical complications involving (but not limited to) reproductive, skeletal, renal, cardiovascular, and central nervous systems (Nattiv et al., 2007). Specifically, potential issues arising from inadequate nutrition in dancers include insufficient peak bone mass and menstrual dysfunction (Kaufman et al., 2002; Warren, Brooks-Gunn, et al., 2002). Moreover, it has been suggested that very lean dancers are more prone to injury than less lean counterparts (Benson et al., 1989). Whilst these concerns have received attention in the literature, much more research involving the clinical implications of energy deficiency (determined using accurate and precise measurement techniques) is required in dance populations.

It is important to note that the first experimental study investigated one specific and unique dance subculture; contemporary dance. Similarly, studies 2-4 investigated the muscle damage response to a dance protocol representative of contemporary dance, or following a repeated-sprint protocol that was shown to elicit a similar response to this exercise. Just as there are many team sports (hockey, soccer and

rugby for instance), there are many forms of dance each with different characteristics and demands across different levels. For instance, while there have been no reported differences at university level, professional modern dancers demonstrate higher oxygen uptakes compared to professional ballet dancers (Chmelar, 1988). However, Wyon et al. (2002) reported that there were no differences in oxygen uptake and heart rate responses to a dance class between university, graduate, and professional contemporary dancers, and that these results were similar to previous research in ballet dancers. Moreover, no significant differences in quadriceps and hamstring peak torque between student and professional ballet and contemporary dancers has been observed (Chmelar, 1988), while others have reported that contemporary dancers have higher muscular endurance compared to ballet dancers (Thomas, 2003). The reader is directed to a systematic review which has included comparisons in components of fitness between contemporary and ballet dancers (Angioi, Metsios, Koutedakis, & Wyon, 2009). Finally, time motion and video analysis has demonstrated significant differences in exercise intensity, changes in direction, and discrete skills between ballet and contemporary dance; which, according to the authors, would have implications on energy systems utilised and on local muscle damage (Wyon et al., 2011). Though there are some inconsistencies in the literature, the variety of dance genres means that arriving at all-encompassing conclusions and recommendations based on one style are challenging. Indeed, given the reported differences in body composition (Liiv et al., 2013) and the aforementioned fitness levels and physiological requirements between genres, it is likely that there are discrepancies between TEI and TEE, the muscle damage response, and ultimately the efficacy of nutritional interventions amongst dance styles. Therefore, the results of this research may be limited to female contemporary dancers, and future work should look to determine whether the findings are transferable to other dance populations. Additionally, it would be valuable to identify whether male dancers are at equal risk of energy imbalance. Certainly, evidence suggests that equal proportions of female and male dancers are susceptible to disordered eating (Nordin-Bates et al., 2011), providing a firm justification for such research to be conducted in future.

This thesis incorporated a variety of dependent variables to assess EIMD and recovery, however the mechanisms of action responsible for the observed effects

could not be precisely determined from these findings. Further research using a greater variety of biomarkers is required to understand the detailed effects of MC and WPH ingestion on muscle damage, oxidative stress, and inflammatory responses. Certainly, following MC supplementation, it remains unclear whether it is oxidative stress and/or inflammation (or indeed alternative mechanisms) that are responsible for the attenuated responses previously reported. Similarly, though accelerated recovery with WPH is thought to be as a result of an improved protein balance and increased MPS, no study has yet measured these variables following ingestion of WPH in EIMD situations. In the same vein, a more holistic approach to the assessment of recovery is warranted in future research - for example, the inclusion of measures of mood, sleep and psychological wellbeing. Indeed, some preliminary evidence that requires further substantiation and further investigation, suggests that MC supplementation improves sleep quality and duration (Howatson, Bell, et al., 2012), and that WPH supplementation can enhance sense of performance capacity (Hansen et al., 2015).

As discussed in the preceding section, there are a number of limitations of this work with regards to the ecological validity of the findings. It was important to provide rigorous experimental control to minimise the influence of confounding variables (for instance exercise and dietary restrictions) and to provide proof-of-concept in regards to the efficacy of MC and WPH treatments in female dancers. This provides a strong foundation for future work to explore these interventions in real-world situations. This might incorporate the examination of their use following multiple exercise bouts and/or on consecutive days, preferably in 'field-based' conditions, to provide a more applied paradigm that is representative of typical dance training. In addition, future work examining efficacy of nutritional interventions on exercise recovery in female dancers, should consider not controlling habitual dietary intake; rather it should be monitored alongside measures of free-living energy expenditure. Indeed, important questions regarding whether energy balance influences EIMD and/or the efficacy of nutritional interventions remain to be answered.

Though beyond the scope of this thesis, the proposal that interventions designed to reduce muscle damage can impair adaptive responses warrants discussion. This could have implications when considering long-term training programmes and is a critical question that has recently captured attention in the literature. The

predominant mechanism of action that has been suggested for the role of MC in ameliorating EIMD and accelerating recovery is the potential to increase antioxidant availability in order to combat free radical production associated with muscle damage and the secondary inflammatory response. While WPH ingestion is primarily thought to mitigate EIMD and accelerate recovery through increases in MPS, protein hydrolysates have also been reported to exhibit antioxidant properties (Peng et al., 2009). However, the attenuation of muscle damage, particularly of the secondary responses to exercise, has been implicated in reducing subsequent physiological adaptation by influencing recovery processes such as protein synthesis (Mikkelsen et al., 2009; Trappe et al., 2002; Urso, 2013) and cell signalling (Gomez-Cabrera, Ristow, & Vina, 2012). While evidence to suggest that antioxidant supplementation does not affect the adaptive effects of exercise has emerged (Mikkelsen et al., 2011; Paulsen et al., 2010; Trappe et al., 2011) the specific influence of MC and WPH supplementation on physiological and functional adaptation has yet to be investigated. Nevertheless, it is important to note that adaptation is not always a priority; indeed, in dance populations, optimal recovery and maintaining an ability to perform on a daily basis is often the primary goal. Consequently, there may be a role of these nutritional strategies in many situations which require dancers to perform on multiple occasions in a short period of time; where maximising recovery is essential for maintaining optimal performance.

The final recommendation for future research directions arising from this work relates to the need to increase the perceived importance and impact of dance nutrition in the dance world. The expectation to maintain a lean body type in dance is well-established and is an inherent part of dance culture. The dancer may commit many aspects of their lives in their determination to achieve the accepted aesthetics, and this easily carries to food (Sandri, 1993). The constant concern regarding energy intake has been described as being ‘built-in’ to the life of a dancer (Cohen et al., 1985). This is exacerbated by the fact that the public, who effectively subsidises the art, also have expectations regarding a dancer’s aesthetic appearance (Bonbright, 1989). As a result, it is argued that rather than a pathological response; dancers’ desire for the appearance of ultra-leanness might be a conditioned one (Calabrese et al., 1983). Consequently, it is difficult to challenge these enduring standards, particularly if it interferes with the strict traditions of master teachers, elite schools,

employees, and therefore the professional success of a dancer. Certainly, concerns relating to dancers' nutrition have been recognised for many years, yet unfortunately remain largely unchanged.

Alternatively, development in current dance nutrition practice may be best achieved through improved education. Lack of nutritional knowledge is one of many factors which may also contribute to low energy intakes observed in dancers. Interestingly, Wyon, Hutchings, Wells, and Nevill (2014) state that it is difficult to determine whether it is dietary restriction or indeed nutritional knowledge, which overrides nutritional intake in dancers. Indeed, these authors reported that dancers with disordered eating display lower levels of nutritional knowledge (Wyon et al., 2014). Therefore, it is strongly advised that nutritional education is embedded in dance instruction, particularly in regards to the importance of energy balance in periods of increased training and performance demand given that chronic energy deficiency can result in substantial and long-lasting health implications. Future research should seek to develop current understanding regarding dancers' nutritional knowledge and behaviours towards food, in order to determine where and how such education programmes could be implemented.

It is equally important for performance nutritionists to recognise and accept the current and likely irrevocable aesthetic demands of dancers. A lack of understanding perhaps explains why only a small percentage of dancers receive dietary advice from professional specialists (Koutedakis, Pacy, Carson, & Dick, 1997), and the precedent for ignoring advice is reported to be widely established (Sandri, 1993). Indeed, a dancer may sooner desert the nutritionist or dietician than the expected aesthetics and their resolve to achieve it (Sandri, 1993). Thus, dance nutrition should be sensitive to the dancers' predicament of achieving optimal nutrition whilst requiring consideration of weight control. The delivery and dissemination of dance nutrition education should therefore reflect this and offer creative application of methods of weight management, without compromising the professional ethics and integrity of the nutritionist. Moreover, given that current nutrition practices and aesthetic ideals are embedded in dance culture, nutritional recommendations may be best received if they are practical and relatively simple to adopt in a dancer's diet. Therefore, the impact of this work could be wide-reaching; particularly in regards to the beneficial effects observed with MC and WPH supplementation.

7.5 Conclusion

In summary, through four progressive research studies, the findings of this course of investigation indicate that, 1) there is a prevalence of energy deficiency in pre-professional female contemporary dancers; particularly during periods of scheduled dance training, 2) female dancers are at risk of exercise-induced muscle damage following both dance and sprint-type exercise and experience the associated negative symptoms for several days, 3) tart Montmorency cherry and whey protein hydrolysate supplementation are able to attenuate damage and accelerate recovery following muscle-damaging exercise to some extent in female dancers.

Notably, this work contributes to the literature with new information regarding the beneficial effects of tart Montmorency cherry and whey protein hydrolysate supplementation for exercise recovery in female dancers; in accordance with previous literature in male and mixed sex groups following a variety of exercise paradigms. In terms of application, this work therefore provides support for the use of tart Montmorency cherry and whey protein hydrolysate supplementation in the day-to-day life of a dancer, as realistic and practical additions to their dietary routines in order to promote exercise recovery as well as improve energy balance.

The popularity and scale of dance participation demonstrates the potential expansive impact that such dance-related research can achieve both in the national and international dance communities. However, while the field of dance medicine and science has emerged over the last 40 years and is now an established area of research, much more research in dancers is warranted. Certainly, it is evident that this population would benefit from population-specific studies to offer evidence-based guidance, as well as improved nutritional education. Yet currently, the advancement of new information predominantly originates from sports science and medicine; where dancers are rarely included in its purview. Both fields of sport science and dance science should work to redress the imbalance of evidence that currently exists in the literature. Indeed, dance and sport specialists should appreciate the inherent similarities between these athletic populations, or else, as suggested by Kleinman (1992), ‘the rich soil, capable of nourishing both sport and dance scholarship and practice, will continue to remain uncultivated.’

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Appendices

Appendix A

INFORMED CONSENT FORM

TITLE OF PROJECT:

Participant ID Number:

Principal Investigator: MEGHAN BROWN

Please tick where appropriate

I have read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐
I would like to receive feedback on the overall results of the study at the email address given below. I understand that I will not receive individual feedback on my own performance. Email address……	☐

Signature of participant..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Signature of researcher..... Date.....……..

(NAME IN BLOCK LETTERS)

INFORMED CONSENT FORM : REMOVAL AND STORAGE OF TISSUE

TITLE OF PROJECT:

Participant ID Number:

Principal Investigator: MEGHAN BROWN

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood	For analysis of creatine kinase and high-sensitivity C-reactive protein	Venepuncture

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste

Other

If other please specify.....

I consent to the University distributing this tissue to partners in this research study, outside of the University, for further testing (please tick the box if you agree). ☐

Signature of participant.....
Date.....……..

Signature of researcher.....
Date.....……..

Appendix B

THE HEALTHIER DANCE PRACTICE NATIONAL SURVEY

Participant ID: _____

Dancers and dance students questionnaire.

CONFIDENTIAL

For the purpose of this questionnaire, the definition of *injury* is 'a physical problem deriving from stress or other causes to do with performance, rehearsal, training, touring or the circumstances of dance life, which affects your ability to participate fully in normal training, performance or physical activity'.

Where asked to indicate numbers of injuries sustained you should count each NEW incidence of injury, even if you have incurred an injury more than once in the same area.

Please try to answer all questions; tick the answer or write where appropriate (capitals please).

1. **Are you currently** **a.** a dance student ☐
   b. a professional dancer ☐

2. **What is** **a.** your gender male ☐ female ☐
   b. your age _____
   c. your ethnic origin _____

3. **Where did you train / are you training now?** Please list if more than one

Name of school or college	**and when?** dates

4. **What age were you when you entered full-time training?** _____

5. **How many years have you been dancing regularly (ie for more than 10 hours a week)?**
   number of years _____

6. **On average how many months a year are you contracted as a dancer?**

   a. 8 months or more ☐

   b. 6-8 months ☐

   c. 3-6 months ☐

   d. less than 3 months ☐

7. **What is your main dance form?**

   a. Afro/Caribbean ☐

   b. Classical Ballet ☐

   c. Contemporary Dance ☐

   d. Jazz ☐

   e. Musical Theatre ☐

   f. South Asian ☐

   g. Tap ☐

   h. Other please specify _____

8. **How many hours on average do you do a week of …**

   a. technical classes number of hours _____
   b. rehearsal number of hours _____
   c. performance number of hours _____
   d. body conditioning number of hours _____
   e. strength training number of hours _____
   f. fitness training (cardiovascular workout) number of hours _____
   g. other number of hours _____
   please specify _____

9. **How many hours of sleep a night on average do you …**

   a. need number of hours _____
   b. get number of hours _____

10. **On average, how many days off (ie time off for yourself) per week do you get altogether?**

    number of days per week_____

11. **Do you warm up… (ie at least 10 minutes of pulse raising activity, joint mobilisation and short stretches)** Tick as many boxes as appropriate

	Yes	**No**	**If yes, for how long?**
a. before class	☐	☐	number of minutes _____
b. before rehearsal	☐	☐	number of minutes _____
c. before performance	☐	☐	number of minutes _____

12. **Do you cool down… (ie at least 10 minutes of pulse lowering, re-mobilisation and stretching)** Tick as many boxes as appropriate

	Yes	No	If yes, for how long?
a. after class	☐	☐	number of minutes _____
b. after rehearsal	☐	☐	number of minutes _____
c. after performance	☐	☐	number of minutes _____

13. **Is time set aside in your school / company to…**

	Yes	No
a. warm up	☐	☐
b. cool down	☐	☐

14. **In the last 12 months, as a result of a dance injury, how many days have you been unable to …**

a. do a full class number of days _____
b. rehearse number of days _____
c. perform number of days _____
d. If the injury was longer-term, how long have you been unable to participate in all three?
number of months _____ weeks _____

15. **Have you had any of the following injuries in training, rehearsal and / or performance in the last 12 months?** Tick as many boxes as appropriate

a. muscle	☐	number _____
b. bone	☐	number _____
c. joint / ligament	☐	number _____
d. tendon	☐	number _____
e. other	☐	number _____ please define _____

16. **If you did have injuries in the last 12 months, where were the sites of injury?** Tick as many boxes as appropriate

a. arms / hands	☐	number _____
b. shoulders	☐	number _____
c. neck	☐	number _____
d. upper back	☐	number _____
e. lower back	☐	number _____
f. ribs	☐	number _____

g. pelvis ☐ number _____

h. groin ☐ number _____

i. hips ☐ number _____

j. thighs (inc. quad / hamstring) ☐ number _____

k. knees ☐ number _____

l. lower legs ☐ number _____

m. ankles ☐ number _____

n. feet ☐ number _____

17. **What type of professional help did you initially have for the injuries?** If more than one please number the boxes in the order you approached them

a. physiotherapist ☐

b. general practitioner ☐

c. specialist / consultant ☐

d. osteopath ☐

e. chiropractor ☐

f. other ☐ please specify _____

18. **Did you seek any other help to aid rehabilitation after these injuries?** If yes, tick as many boxes as appropriate

a. masseur ☐

b. acupuncturist ☐

c. dietician ☐

d. counsellor ☐

e. psychologist ☐

f. Pilates ☐

g. other ☐ please specify _____

19. **Who paid for the treatment of your last injury?** Tick all that apply if the cost was shared

a. myself ☐

b. medical insurance company ☐

c. employer / school ☐

d. NHS ☐

20. Can you give the rough cost *to you* of all treatments of injury over the last 12 months?

£ _____

21. What do you think was the cause of these injuries? Please take into account all your injuries in the last 12 months and tick as many responses as are applicable

a. fatigue ☐

b. overwork ☐

c. unsuitable floor ☐

d. cold environment ☐

e. insufficient warm up ☐

f. new / difficult choreography ☐

g. different repertory ☐

h. repetitive movements ☐

i. partnering work ☐

j. incorrect technique / training ☐

k. ignoring early warning signs ☐

l. recurrence of old injury ☐

m. inadequate diet / hydration ☐

n. set / props ☐

o. costume / shoes ☐

p. rehearsal schedule ☐

q. other ☐ please explain briefly ____

22. What do you do if you suspect an injury? Please tick all that apply

a. seek professional medical treatment (eg physiotherapist, GP etc.) ☐

b. tell someone else (eg teacher / director) ☐

c. take own preventative steps ☐

d. take pain killers ☐

e. continue to dance, but carefully ☐

f. ignore it ☐

g. hide it ☐

h. other ☐ please specify ___

23. Who has the most influence in guiding your return to activity?

a. medical professional ☐

b. company staff ☐

c. teacher ☐

d. yourself ☐

e. other ☐ please specify _____

24. Are you currently a smoker? Yes ☐ No ☐

25. How many cigarettes do you smoke a day? number _____

26. If you previously smoked …

a. how many cigarettes did you smoke a day? number_____

b. for how many years did you smoke?

Include all smoking periods if you have given up more than once

number of years ____ months____

c. how long ago did you stop? number of years ____ months____

27. How many units of alcohol do you drink a week on average?

(1 unit = a glass of wine, measure of spirit, half a pint of beer)

number of units _____

28. Are you aware of the implications to your fitness and performance of…

	Yes	**No**
a. smoking	☐	☐
b. drinking alcohol	☐	☐
c. abuse of drugs	☐	☐

29. If you currently follow any particular dietary or nutritional plan, please state briefly what it is:

a. vegan (no meat, fish, or dairy products) ☐

b. vegetarian (no meat or fish) ☐

c. weight reducing ☐

d. weight gaining ☐

e. other ☐ please specify_____

_____

30. Do you take any nutritional supplements? Yes ☐ No ☐

a. vitamins ☐

b. iron ☐

c. calcium ☐

d. other ☐ please specify _____

31. Where do you take advice on nutrition from?

a. an accredited dietician ☐

b. GP ☐

c. company / school staff ☐

d. friends ☐

e. media / literature ☐

f. other ☐ please specify _____

32. Do you feel you have ever had an eating problem? Yes ☐ No ☐

If yes, please give details if you would like to _____

_____

33. What is…

a. your height ___ ft ___ ins or _____cm

b. your weight ___ st ___ lbs or _____kg

c. the lightest your adult (over 17 yrs) weight has ever been _____

d. what age were you when your adult weight was the lightest _____yrs

e. the heaviest your adult (over 17 yrs) weight has ever been _____

f. what age were you when your adult weight was heaviest _____yrs

<u>WOMEN ONLY (men go to question 40):</u>

34. At what age did your periods start? _____yrs

35. Have you ever taken the oral contraceptive pill?

Yes ☐ No ☐ (if no go to question 37)

36. For how long have you taken / did you take the pill?

a. 0 - 6 months ☐

b. 6 months - 1year ☐

c. 1 - 2 years ☐

d. 2 - 3 years ☐

e. 3 - 4 years ☐

f. 4 - 5 years ☐

g. 5+ years ☐

37. When not on the pill, are your periods regular (occurring every 25-35 days)?

Yes ☐ No ☐

38. Have your periods ever stopped for more than 6 months?

Yes ☐ No ☐

If yes, have you sought medical advice? Yes ☐ No ☐

39. What is the longest gap you have had between your periods?

months _____ years _____

40. Have you ever experienced any of these in the last 12 months?
Tick as many boxes as are applicable

a. general anxiety ☐

b. tension with people ☐

c. performance anxiety ☐

d. depression ☐

e. stress due to external factors ☐
(eg bereavement, moving house)

f. eating problems ☐

g. over-use of alcohol / drugs ☐

h. general low self-confidence ☐

i. sudden drop in self-confidence ☐

j. consistent difficulty in concentrating ☐

k. constant tiredness ☐

l. burnout ☐

m. feeling pressure to return to performance earlier than advised ☐

n. English as a second language ☐

41.

a. What do you feel are the main pressures of life as a dancer? _____
_____

b. How do you cope with these? _____
_____

42. Have you ever made use of a professional counsellor / psychologist to talk through personal or professional difficulties?

	Yes	**No**
a. as a student	☐	☐
b. as a professional dancer	☐	☐

If yes, how many sessions did you have? number _____

Did you find it helpful? Yes ☐ No ☐

43. Do you have access to a counsellor / psychologist now if you want one?

Yes ☐ No ☐

44. If no, would it be helpful to you to have access to an independent professional to whom you could talk?

Yes ☐ No ☐

45. Do you feel that your vocational (pre-professional) training prepared / is preparing you well for life as a dancer in terms of…

	Yes	**No**	**More or less**
a. dance technique	☐	☐	☐
b. health and fitness	☐	☐	☐
c. psychological readiness	☐	☐	☐
d. career advice	☐	☐	☐
e. understanding of the dance profession	☐	☐	☐

46. If not, which areas do you feel would have helped / help to improve your performance and long term career prospects?

_____

47. How have you started to plan for your career / life beyond performing?

a. consulting Dancers' Career Development ☐

b. talking to a counsellor ☐

c. following a course of study ☐

d. developing other practical skills ☐

e. planning to have a family ☐

f. pension scheme ☐

g. other ☐ please specify __
_____

48. If you had a magic wand and could change one thing that would do most to promote the health, well-being, excellence and longevity of dancers – what would it be?

_____
_____

Signature of participant: _____ Date:_____

Signature of test supervisor: _____

Appendix C

MENSTRUAL CYCLE QUESTIONNAIRE

Participant ID:_____

Please answer the following questions by circling the appropriate response:

1. Age at menarche (*first menstrual cycle*) _____ years _____ months
2. Do you have periods? YES NO

   If NO when was your last period? _____

   If YES how regular are they? 4-9 per year every month

3. How long is your menstrual cycle, from day 1 of bleeding to day 1 of the next bleed? ____days *(if using oral contraceptives this is 28 days)*

4. How many days does your menstrual (*blood*) flow last? ____days
5. What day of your cycle are you on today?

   day 1 = first day of pill (if using oral contraceptives)

   or day 1 = first day of bleeding

   1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

   18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

6. Do you use contraceptive pills or use any other form of feminine contraception?

   YES NO

If YES please complete the following:

Brand/form:_____

Duration (years/months):_____

When (time of day) if applicable:_____

Do you take pill packs back-to-back? YES NO

If YES how often? Please give details:

_____

_____

Any other details:

_____

_____

Signature of participant: _____ Date: _____

Signature of test supervisor: _____

Appendix D

THREE FACTOR EATING QUESTIONNAIRE – R18

Participant ID: _____

Please answer the following questions by circling the response that is most appropriate to you.

1. I deliberately take small helpings as a means of controlling my weight.
   definitely true / mostly true / mostly false / definitely false
2. I consciously hold back at meals in order to not gain weight.
   definitely true / mostly true / mostly false / definitely false
3. I do not eat some foods because they make me fat.
   definitely true / mostly true / mostly false / definitely false
4. How frequently do you avoid 'stocking up' on tempting foods?
   almost never / seldom / usually / almost always
5. How likely are you to consciously eat less than you want?
   Unlikely / slightly likely / moderately likely / very likely
6. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?
   1 / 2 / 3 / 4 / 5 / 6 / 7 / 8
7. When I smell a sizzling steak or a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.
   definitely true / mostly true / mostly false / definitely false
8. Sometimes when I start eating, I just can't seem to stop.
   definitely true / mostly true / mostly false / definitely false
9. Being with someone who is eating often makes me hungry enough to eat also.
   definitely true / mostly true / mostly false / definitely false
10. When I see a real delicacy, I often get so hungry that I have to eat right away.
    definitely true / mostly true / mostly false / definitely false
11. I get so hungry that my stomach often seems like a bottomless pit.
    definitely true / mostly true / mostly false / definitely false

12. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.
    definitely true / mostly true / mostly false / definitely false
13. I am always hungry enough to eat at any time.
    definitely true / mostly true / mostly false / definitely false
14. How often do you feel hungry?
    only at mealtimes / sometimes between meals / often between meals / almost always
15. Do you go on eating binges though you are not hungry?
    never / rarely / sometimes / at least once a week
16. When I feel anxious, I find myself eating.
    definitely true / mostly true / mostly false / definitely false
17. When I feel blue, I often overeat.
    definitely true / mostly true / mostly false / definitely false
18. When I feel lonely, I console myself by eating.
    definitely true / mostly true / mostly false / definitely false

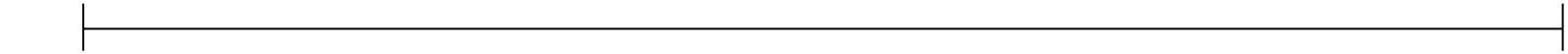
Signature of participant: _____ Date: _____

Signature of test supervisor: _____

Appendix E

MUSCLE SORENESS VISUAL ANALOGUE SCALE

After a 90 ^{0} squat how sore are your muscles?



No soreness Unbearably sore